MUTABLE CHARACTERS OF *DROSOPHILA VIRILIS*

I. REDDISH-ALPHA BODY CHARACTER *

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INTRODUCTION

In a preliminary report on the behavior of the "reddish" body-color character (DEMEREC 1926a) it was suggested that the unusual results obtained in different experiments could readily be explained by the assumption that reddish mutates frequently to the wild-type. This assumption has been made still more probable by the results obtained in recent studies on reddish. It is also supported by the work on the behavior of the miniature-α wing character (DEMEREC 1926b) and the magenta-α eye-color character (DEMEREC 1927a), both of which revert

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in the same way as reddish does. In this paper an endeavor will be made to give a detailed account of the results obtained only in experiments with reddish. The details of the experiments with miniature-α and magenta-α will be presented in subsequent papers of the same series.

NOMENCLATURE

To designate a character showing frequent heritable changes, the terms "mutable", or "frequently mutating" will be used. The process of change will be called "mutation", and also "reversion"; since in the case of reddish it was observed that the change occurs only in one direction, namely from reddish to wild-type. Thus the term "mutation" and its derivatives are applied both to the reversion of a mutant character to the wild-type, as well as to the origin of the mutant character from the wild-type.

The Greek alphabet is used to distinguish mutable from constant allelomorphs.

The symbol *re*-α will be applied to the mutable reddish. Since reddish is an allelomorph to yellow, and yellow has been known longer (METZ 1916) than reddish the strictly correct symbol for the mutable reddish would be $y^{re-\alpha}$. As a matter of convenience, however, the shortened symbol *re*-α will be used in this paper.

THE ORIGIN OF REDDISH

The character called reddish was found twice in matings involving in both cases the stock line, concave, approximated, telescoped. The period of seventeen months elapsed between the first and the second origin. The first reddish is mutable; and will be called reddish-α while the second reddish is constant, and will be called reddish-1. The second reddish was found by Miss MILDRED S. MOSES, to whom the writer is indebted for permission to use the data relating to the origin of reddish-1 and also for the use of flies bearing that character in experiments.

The origin of reddish-alpha

In experiments planned to test the effect of centrifugal force on the behavior of sex chromosomes, a cross was made in which a centrifuged yellow vermilion female was crossed with a sepia scute short rough male; and among a total of 104 offspring, a female was found which had several extra veins on both wings. To determine the inheritance of this character, the offspring of the female were tested for two generations. When it was found to be a dominant autosomal character, a cross was made to

determine the chromosome in which it was located. For that purpose, a concave approximated telescoped female was crossed with a sepia vermilion branched-veins male; and five of the F_1 females were backcrossed with concave approximated telescoped males. In one of the backcrosses, half of the males had a reddish body; and in another backcross, among a total of 217 flies, one reddish male was found. The remaining three backcrosses gave no reddish flies.

Subsequent tests showed that the extra-veins character was a recurrence of branched, the latter having been already described (METZ, MOSES and MASON 1923). Reddish flies, therefore, originated from the cross between a newly found branched and concave approximated telescoped flies. They were found on January 8, 1924, in two separate matings. In one case, half of the males were reddish; indicating that the female parent (P_1) was heterozygous for the character. It is possible that in this case the mutation occurred late in the P_2 female, affecting only a few germ cells, one of which was transmitted to the P_1 female; or that the mutation occurred early in the development of the P_1 female, making it heterozygous for reddish; or that the mutation affected a few P_2 males. In the second mating noted above, where only one reddish male was found among 217 flies, the possibility is not excluded that it came as a contamination from the other mating, where many such males were found. It is, however, at least as probable that it originated as an independent mutation, affecting only a few germ cells of the P_1 female, as that it came as a contamination.

All reddish flies used in the experiments were progenies of the mating which gave half of the males reddish. The single reddish male found in the other mating was not used in breeding.

The origin of reddish-1

To locate a certain newly found character, which turned out to be another allelomorph of branched, Miss M. MOSES made a cross between the character in question and concave approximated telescoped flies from the stock culture. On June 16, 1925, in one of two backcrosses, one reddish male was found among a total of 145 flies. Reddish-1, therefore, originated, similarly to reddish-α, in a backcross involving the newly found character branched and concave approximated telescoped.

Breeding experiments with the original reddish-1 male showed that it was heterozygous for the characters concave approximated and telescoped. Since there were no reddish-α flies which could have had a

similar genetic constitution, at that time in the laboratory, it is unlikely that the first reddish-1 male was a contamination from reddish-α. The results of crosses between reddish-α and reddish-1, which will be discussed later, made the possibility of contamination having occurred still more improbable.

Attempts to obtain other reddish mutants

Both reddish-α and reddish-1, as already stated, originated in matings involving branched and concave approximated telescoped characters. The character, branched, used in these different crosses, came from unrelated sources; but the concave approximated telescoped flies were in both cases taken from the same stock line. It is probable that it was due to chance that both of the reddish mutations occurred in the progenies of crosses where flies from a particular line were used; but there is a possibility that the line concave, approximated telescoped, used in both cases, was potentially mutable to reddish. To test that possibility, the cross between concave approximated telescoped and branched flies was repeated seven times; and 217 F_1 females from these crosses were backcrossed with concave approximated telescoped males. Among 16,890 offspring of these backcrosses no reddish flies were found. These results indicate that if the concave approximated telescoped line mutates to reddish at all, the frequency of such mutations is very low.

DESCRIPTION

The body, wings, legs, bristles and hairs of the reddish flies are reddish-yellow; and contrast with the dark gray color of these parts of the body in wild-type flies, and the yellowish gray color in yellow flies. The color of the flies varies with the age and condition of the cultures. Extreme variations of reddish and yellow might overlap in color of the body; but no case has been observed where reddish and yellow could not be distinguished by the color of the hairs and bristles, which is yellowish gray in reddish and gray in the yellow flies.

Reddish-α and reddish-1 can not be distinguished.

PARTIAL MAP OF THE SEX CHROMOSOME

Several sex-linked characters were extensively used in the experiments which will be discussed in this paper. For a better understanding of the data the partial map of the X-chromosome given in figure 1 may be useful. The majority of the characters mentioned in the map have already been described (METZ, MOSES AND MASON 1923). The descriptions

of some of them, however, are not yet published, since the characters were only recently found by either Dr. C. W. METZ or Miss MILDRED S. MOSES, to whom the writer is indebted for the use of the material.

INHERITANCE OF REDDISH-ALPHA

Reddish-α was found to be a sex-linked recessive character. The crosses between reddish females and not-reddish males always gave not-reddish females and reddish males; and from the matings where not-reddish females and reddish males were used, all the F_1 generation was not-reddish.

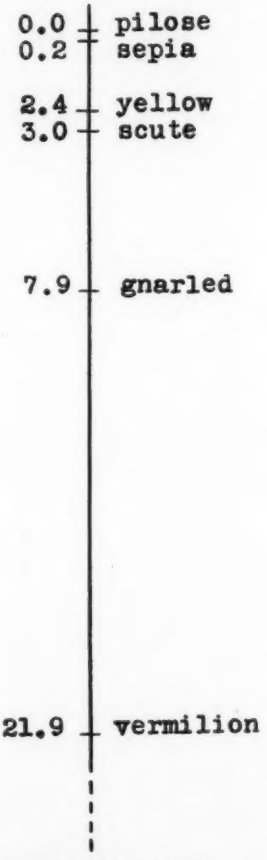


FIGURE 1.—Map of left end of sex-chromosome.

Allelomorphism to yellow

If two allelomorphs are recessive to wild-type, the F_1 generation when they are crossed is usually intermediate. It could not be of the wild-type, since it is assumed that allelomorphs are located in homologous loci, and only two of them can be present in the usual type of inheritance. Two non-allelomorphic recessive characters on the other hand, always give a wild-type F_1 generation. In the F_2 generation of the crosses between two allelomorphs, only parental and F_1 types are expected. Bar in *Drosophila melanogaster* (STURTEVANT 1925) is the only case where two allelomorphs combine in one chromosome, and that rarely. It has often been pointed out that, in all cases where several allelomorphs are known, they affect the same character. The difference between them is rather quantitative than qualitative; therefore the test for allelomorphs should show (a) that the F_1 generation from a cross between two of them is not of the wild-type; (b) that they do not recombine in the F_2 generation (c) that their position in respect to other genes is the same (except in case of such chromosomal abnor-

malities as duplication or inversion), and (d) also that they affect the same character.

Reddish and yellow fulfill these requirements. When intercrossed,

TABLE 1

Data from the crosses with "constant" reddish-α, showing linkage relations.

CONSTITUTION OF F_1 ♀'s			r_a / $s_a y s_c v$	r_a / $pl\ s_c$ 1st experiment	r_a / $pl\ s_c$ 2nd experiment	r_a / $pl\ s_c$ 3rd experiment	r_a / $pl\ s_c$ Total	$s_a r_a$ / $pl\ s_c$	$pl r_c$ / $s_a y g_n$	$r_a g_n$ / $s_a y s_c v$ 1st experiment	$r_a g_n$ / $s_a y s_c v$ 2nd experiment	$r_a g_n$ / $s_a y s_c v$ Total
F_1 pedigree number			969	505	710	711	..	966	967	968	970	..
No. of F_2 matings			23	6	10	7	23	15	16	22	14	36
Total ♀			1972	500	434	301	1235	1222	1349	1812	978	2790
♂	0*	a†	849	268	173	137	578	533	530	610	274	884
		b	664	207	134	86	427	593	471	537	347	884
	1	a	26	4	1	1	6	2	1	17	7	24
		b	22	1	4	3	8	1	..	13	12	25
	2	a	6	6	1	..	7	18	6	2	1	3
		b	7	3	2	..	5	10	4	3	1	4
	3	a	193	..	..	..	..	1	21	43	19	62
		b	151	..	..	..	..	6	33	36	13	49
	4	a	..	..	..	..	..	..	..	103	48	151
		b	..	..	..	..	..	..	..	113	49	162
	1–3	a	..	..	..	..	..	..	..	..	..	..
		b	1	..	..	..	..	..	..	..	..	1
	1–4	a	..	..	..	..	..	..	..	1	..	1
		b	..	..	..	..	..	..	..	1	1	2
	Total		1919	489	315	227	1031	1164	1066	1479	772	2251

* In this and the following tables "0" signifies non-crossover classes; "1" classes resulting from crossing over in the first region, etc.

† The class having r_a is always placed in column *a*.

F_1 females were always yellow or nearly so. Since the color of the body of the flies varies with age and environmental conditions, it is difficult to detect slight differences due to the genetic constitution; and it is not

TABLE 2

Data from the crosses $r_e/s_e\ y\ s_c\ v$ ♀ × $s_e\ y\ s_e\ v$ ♂.

Number of experiments Number of matings			32 184
♀'s	r_e and y		11053
	Reversions	+ v $v\ s_c$	138 22 18
	Total		11231
♂'s	0	r_e $s_e\ y\ s_e\ v$	4067 3648
	1	$s_e\ r_e$ $y\ s_c\ v$	92 75
	2	$r_e\ s_c\ v$ $s_e\ y$	25 37
	3	$r_e\ v$ $s_e\ y\ s_e$	984 885
	1–3	$s_e\ r_e\ v$ $y\ s_c$	.. 3
	2–3	$r_e\ s_c$ $s_e\ y\ v$	1 ..
	Reversions	+ v s_c	118 30 23
	Total		9988
Total	y and r_e		20870
	Reversions		349

possible to determine with certainty if reddish yellow females are not lighter than homozygous yellow females. If there is any difference between these two types of females it is very slight.

Soon after its discovery, two lines of reddish-α were isolated: one which mutated frequently to wild-type; and one in which reddish-α behaved like a constant character. In the F_2 generations from the crosses between yellow and "constant" reddish-α, only reddish and yellow offspring were obtained; as was to be expected, if these two characters were allelomorphs. In table 1, 11,347 progeny from such crosses are listed. Table 1 gives

TABLE 3

Data from the crosses pl re/s_e y s_e ♀ × s_ey s_e ♂.

Number of experiments			12
Number of matings			98
♀'s	r_e and y		5462
	Reversions	+	68
		s_e	5
		s_e	1
	Total		5536
♂'s	0	r_e pl	2119
		s_e y s_c	2299
	1	r_e	..
		pl s_e y s_c	2
	2	s_e r_e	57
		pl y s_e	37
	3	pl r_e s_c	12
		s_e y	29
	Reversions	pl	51
		pl s_e	5
	Total		4611
Total	r_e and y		10017
	Reversions		130

the data from crosses involving constant reddish-α and pilose, sepia, yellow, scute, gnarled and vermilion, which indicate that reddish-α is located in approximately the same region of the chromosome as yellow.

Finally both yellow and reddish-α are similar in effect, as is the case, as already stated, with all known allelomorphs belonging to one group.

This evidence supports the assumption that reddish-α is an allelomorph of yellow.

Mutability of reddish-alpha

There is, however, a line of reddish-α which does not behave like the line described in the previous section. When reddish from that line is crossed with yellow, F_1 females are yellow, as usual; but in the F_2 generation, in addition to reddish and yellow flies, wild-type flies appear in a variable number. If reddish is an allelomorph of yellow, as the analysis of the data from table 1 indicates, then these wild-type flies are exceptions from the usual behavior of allelomorphs. Or the wild-type flies obtained in crosses between reddish-α and yellow might be crossovers between these two factors; which would indicate that reddish and yellow are not allelomorphs, and that the composition of the F_1 generation in crosses between them was exceptional.

TABLE 4

Data from the crosses $r_\alpha/p_1\ s_\alpha$ ♀ × $p_1\ s_\alpha$ ♂.

NUMBER OF EXPERIMENTS	NUMBER OF MATINGS	TOTAL ♀	♂							TOTAL
			0		1		2		Reversions	
			r_α	$p_1 s_\alpha$	$r_\alpha\ p_1$	s_α	$r_\alpha s_\alpha$	p_1	+	
6	75	5207	2196	1978	31	50	17	27	150	4449

In tables 2, 3 and 4 a summary is given of the data from crosses involving reddish-α and pilose, sepia, yellow, scute and vermilion. From table 2, which summarizes crosses between reddish-α and sepia yellow scute vermilion, it can be seen that the not-reddish and not-yellow classes (listed under the heading of reversions) offer an obstacle to the usual analysis of the data. As already pointed out in an earlier paper (DEMEREC 1926); if all classes are considered together, it is not possible to locate reddish in the region of the chromosome where it belongs. When an analysis is made in that way, reddish does not fit into the linear arrangement of the genes. It is not probable that crossing over is responsible for the occurrence of these not-reddish and not-yellow classes; since double crossovers between the sepia and scute regions would be required to obtain the observed combinations. The distance between sepia and scute is about 3 units, which is much too short to allow any double crossovers, especially as many as there would be if the not-reddish and not-yellow flies were assumed to be double crossovers. The same holds true when the data given in tables 3 and 4 are analysed.

The problem looks much simpler if the analysis is made without the

not-reddish and not-yellow classes. Then reddish behaves in the same way as in the crosses discussed in the previous chapters; that is, as an allelomorph of yellow. In that case, however, it is necessary to account for the origin of the not-reddish and not-yellow classes. An examination of the data given in the columns headed "reversions" in tables 2, 3 and 4, reveals that all these classes have the reddish chromosome, in which the wild-type allelomorph has been substituted for reddish. As already indicated, crossingover can not account for that substitution. The only other probable assumption is that in these cases reddish mutated back to the wild-type allelomorph. That assumption would easily account for the origin of all the observed classes. If it is assumed that reddish is an allelomorph of yellow and frequently reverts to wild-type, then all

TABLE 5

Comparison between reddish and not-reddish classes in crosses with constant and mutable reddish-α.

CROSS	MUTABLE REDDISH						CONSTANT REDDISH			
	r_e		Not r_e		Reversions		r_e		Not r_e	
	Total	Percent	Total	Percent	Total	Percent	Total	Percent	Total	Percent
$r_e \times y v$	223	40.2	251	45.2	81	14.6	479	53.0	424	47.0
$r_e \times p_l s_c$	2062	50.0	1911	46.4	149	3.6	769	56.6	589	43.4
$r_e \times s_c y s_c v$	5024	51.5	4551	46.6	186	1.9	3214	52.8	2877	47.2
$r_e p_l \times s_c y s_c v$	2019	47.2	2203	51.6	51	1.2	551	48.6	583	51.4

wild-type classes from crosses between reddish and sepia yellow scute vermilion could be interpreted as reversions. That would separate them from the remaining classes, and eliminate all the difficulties otherwise encountered in the explanation of the results.

If reddish-α reverts frequently to the wild-type allelomorph, it ought to be expected that, in the experiments involving mutable reddish-α the reddish classes would be in deficiency when compared with the not-reddish classes. Morever, it is to be expected that the deficiency would correspond to the number of reversions. Unfortunately there is a differental viability between reddish and yellow flies, which is still more pronounced if pilose or sepia and scute are present. That prevents a direct comparison between the reddish and not-reddish classes. When, however, the reddish classes from the experiments with mutable reddish are compared with the reddish classes from experiments with constant reddish (table 5), and both of them are compared with the not-reddish classes of the same experiments, it is noticeable that the mutable reddish shows a deficit approximately equal to the number of reversions.

Constancy of reverted-reddish-alpha

To test the behavior of the wild-type flies which originated as reversions from the mutable reddish-α, 42 wild-type males were mated to sepia yellow scute vermilion females. All F_1 females were wild-type, and of 18,811 F_2 offspring, none was reddish. These results indicate that the mutations in the reddish locus occur in one direction only, namely, from reddish to wild-type, and not from wild-type to reddish.

TABLE 6

Distribution of reversions among the progenies of single females which females came from a pair mating.

PEDIGREE NUMBER	TOTAL NUMBER OF FLIES	NUMBER OF REVERSIONS	PERCENTAGE OF REVERSIONS
2881	101	3	2.97
2882	153	0	..
2883	95	1	1.05
2884	118	1	.85
2885	156	3	1.92
2886	118	1	.85
2887	136	12	8.82
2888	136	0	..
2889	33	3	9.09
2890	199	5	2.51
2891	115	4	3.48
2892	115	1	.87
2893	125	1	.80
2894	135	0	..
2987	103	2	1.94
2988	112	3	2.68
2989	55	5	9.09
2990	123	3	2.44
Total	2,128	48	2.26

INHERITANCE OF REDDISH-1

Experiments with reddish-1 indicated that it is an allelomorph of yellow and reddish-α; and also that, unlike reddish-α, it does not revert to the wild-type. In crosses between reddish-1 and yellow, all F_1 females were yellow; and, among 15,103 F_2 progenies, nothing but yellow and reddish flies was observed.

TIME OF OCCURRENCE OF MUTATIONS

Reversions of reddish-α to the wild-type were observed only among the progenies of females heterozygous for reddish-α. Numerous homozygous females were tested, and also several homozygous reddish-α stocks

were kept for many generations; but in no case did any change in reddish appear. Males also always behaved as constant reddish. Numerous F_1 females from crosses of yellow females by reddish males were examined, but none of them were of the wild-type. Reversions to wild-type occurring in reddish-α males would also have been noticed in stocks homozygous for reddish.

Females heterozygous for mutable reddish-α gave numerous reversions. All of these reversions affected the whole fly, indicating that the mutations occurred at the formation of the ova. If mutations occurred in somatic cells, it would be expected that mosaics would develop frequently, which was not the case. Throughout the experiments a close watch was kept for reddish and wild-type mosaics, but none was observed. If they occur at all they must be very rare. This makes negligible the hypothesis that the reversions occurred early in the somatic development.

If reversions of reddish to wild-type occurred in the oogonial divisions, one mutation would affect a group of germ cells, and the result would be that the reverted flies would appear in groups. This was not the case (table 6). In the low mutating line, usually single wild-type flies were obtained from individual females. With increase in the frequency of mutations the number of wild-type flies from single females increased also. The occurrence of reversions in groups, however, was an exception rather than a rule; which makes it improbable that the mutations occurred in the oogonial divisions, and points to the maturation division as the possible seat of the change. A relation was observed between crossing over and the occurrence of mutations, which suggests that both processes occurred at the same time, and supports the assumption that the mutations occurred at the maturation divisions.

In table 4, the data are given from the crosses between mutable reddish-α and pilose scute. From these data it can be seen that reddish mutated in the females which had in one chromosome reddish-α, and in the other chromosome the wild-type allelomorph. From the data given in tables 2 and 3, it is evident that reddish-α mutated in the females which were heterozygous for its allelomorph yellow. The data from table 8 show that reddish-α mutated in reddish-α over reddish-1 females. Reddish-α therefore mutates at the maturation divisions of females having in one chromosome mutable reddish-α, and in the other chromosome one of its allelomorphs; that is, wild-type, yellow or reddish-1. No mutation has been observed in the females homozygous for mutable reddish-α.

Wild-type which originated by a mutation from reddish-α behaves like any other wild-type; that is, it keeps the reddish-α mutating when heterozygous with it. This can be seen from the data in table 7; which

TABLE 7

The F_2 data from a cross between a reddish-α sepia female and a pilose scute vermilion male.

PEDIGREE NUMBER	♀	0		2		3		4		REVERSIONS	
		$se\ r_\alpha$	$pl\ sc\ v$	$pl\ r_\alpha$	$se\ sc\ v$	$se\ r_\alpha\ sc\ v$	pl	$se\ r_\alpha\ v$	$pl\ sc$	se	$se\ v$
2516	47	9	13		1			5	3		
7	102	15	27			1		3	10	1	1
8	47	11	13		1			3	5	1	1
Total	196	36	53		2	1		11	18	2	2

give the F_2 results of a cross between a reddish-α sepia female and a pilose scute vermilion male. This male was a reversion obtained from a reddish-α pilose scute over sepia yellow scute vermillion female.

TABLE 8

Comparison of frequency of mutability in reddish-α/reddish-1 and reddish-α/yellow females.

PEDIGREE NUMBER	NUMBER OF MATINGS	NUMBER OF MATINGS GIVING REVERSIONS	TOTAL		PERCENTAGE OF REVERSIONS
			y and r_α	Reversions	
		Offspring of re-α/re-1 females			
2816	10	3	1160	7	0.6
2817	20	13	2506	24	0.9
2818	1	1	122	1	0.8
2819	5	2	655	5	0.8
Total	36	19	4443	37	0.8
		Offspring of re-α/y females			
2816	3	..	338	..	..
2817	29	17	3022	30	0.10
2818	1	1	93	7	7.0
2819	3	1	324	6	1.8
Total	36	19	3777	43	1.1

To determine if there was any difference in the frequency of mutations between the reddish-α over yellow and reddish-α over reddish-1 females, the same reddish-α male was mated to yellow and reddish-1 females, and the F_2 results of these crosses compared. A summary of the experiments, which were repeated four times, is given in table 8.

They indicate that reddish-α mutates with equal frequency when heterozygous with yellow or with reddish-1.

FREQUENCY OF REVERSIONS

About half of the progenies of females in which reddish-α mutates to wild-type, carry the reddish-α gene; since, as already mentioned, reddish-α can mutate in heterozygous females only. The ratio between

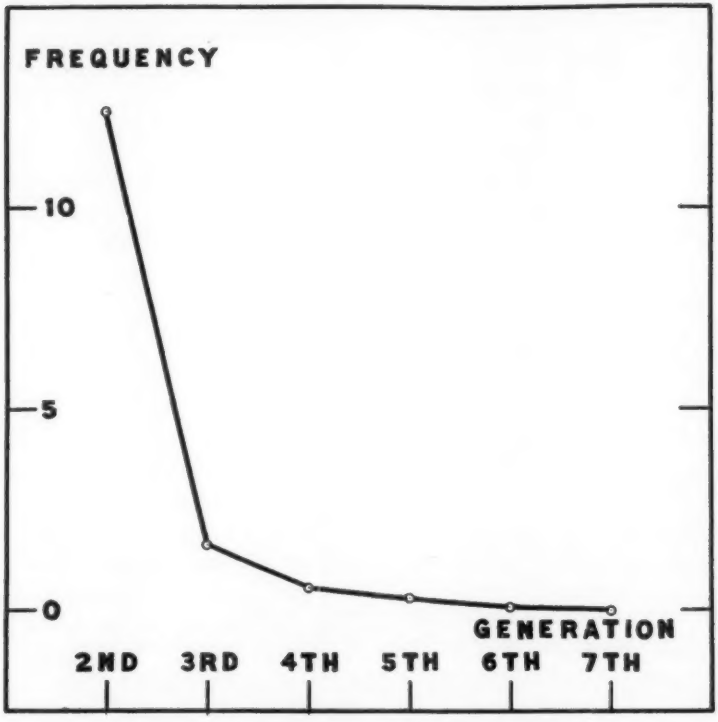


FIGURE 2.—Frequency of reversions of reddish-α to wild-type, immediately after the origin of reddish-α.

the number of reversions and the number of progenies carrying the reddish-α gene would give a measure of the frequency with which reddish-α mutated to the wild-type. Since in many experiments the reddish-α and not-reddish-α progeny had not been counted separately (and in some experiments it was not possible to separate them), the frequency

of reversions throughout this paper is expressed not by the proportion between the number of reversions and the number of progeny carrying the reddish-α gene, but by the proportion between the number of reversions and the total number of the progeny. Since approximately only half of the total number of progeny of heterozygous females was expected to carry the reddish-α gene, the "frequency" of reversions as the term is used in this paper will amount to about half of the actual frequency.

Mutability of reddish-alpha at the time of origin

The progenies of yellow reddish-α females offer the best material for the determination of the frequency with which reddish-α reverts to wild-type; since among these progenies all classes, namely, yellow,

TABLE 9

Frequency of reversions of reddish-α to wild-type, immediately after the origin of reddish-α.

GENERATION	TOTAL NUMBER OF FLIES	NUMBER OF REVERSIONS	FREQUENCY OF REVERSIONS PERCENTAGES*
2nd	241	30	12.4
3rd	9633	152	1.6
4th	8022	48	0.6
5th	2147	7	0.32
6th	11532	5	0.043
7th	4041	0	0.0

* Since the reddish gene was present in half of the gametes only and in calculations total number of flies was used, the frequency of reversions was about twice as large as given in this column.

reddish-α and reversions, can easily be distinguished. When reddish-α was found, one of the first crosses made with it was with yellow. The same cross was repeated for different purposes through many generations. This made it possible to determine the frequency with which reddish-α reverted in different stages of the experiments.

Fig. 2 represents the curve and table 9 gives the actual data on the frequency with which reddish-α mutated to the wild-type during the second to seventh generation after it was found. At that time the matings were made at random, without any selection to increase the frequency of of reversions. As can be seen from both fig. 2 and table 9 the frequency reversions decreased rapidly in succeeding generations; until in the seventh generation no reversion was found among 4041 individuals. In the second generation after the origin of reddish-α, the females heterozygous for reddish-α gave 12.4 percent of reversions; two gener-

ations later, similar females gave 0.6 percent of reversions; and in the sixth generation, among 11,532 offspring, only 5 reversions were observed.

Effect of selection on the mutability of reddish-α

The mutable reddish-α stock was kept up for a long time by back-crossing reddish-α over sepia yellow scute females with sepia yellow scute males. Since there is no double crossing over between sepia, yellow and scute, all yellow females from that cross had the genetic constitution of their mother. It was therefore possible to perpetuate the cross by mating yellow females with sepia yellow scute males from the same culture.

TABLE 10

Effect of selection on the frequency of reversions.

GENERATION	TOTAL NUMBER OF FLIES	NUMBER OF REVERSIONS	PERCENT OF REVERSIONS
1	591	23	3.9
2	196	7	3.6
3	578	6	1.0
4	45	4	8.9
5	180	8	4.4
6	92	2	2.2
7	415	14	3.4
8	491	12	2.4
9	171	3	1.8
10	362	4	1.1
11	795	21	2.6
12	796	25	3.1
13	5731	145	2.5
14	7625	99	1.3
15	5727	276	4.8
16	4890	178	3.6
Total	28.685	827	Average 2.9

Pair matings were always used to propagatè a line. Since reddish-α mutates in heterozygous females only, this was the simplest way to keep a reddish-α stock which was certain to be mutating.

If many pair matings were made from a culture which gave a high number of reversions, the number of reversions in the offspring varied from zero to as high as, or higher than, in the parent culture. It has been shown before that the frequency of reversions decreased rapidly if the matings were made at random. If, however, the matings were made from cultures which gave a high number of reversions, the frequency of reversions did not decrease, but remained more or less constant. That can be seen from table 10, which records the results of an experiment

where selection was carried on for sixteen generations with the purpose of increasing the frequency with which reddish-α mutated. During that time the line was propagated from the cultures which gave the highest number of reversions. By this selection increase in the mutability was not attained, but neither did a decrease occur. The mutability in the line remained approximately the same. In individual cultures, the number of reversions was as high as 25 percent; but in the totals of different experiments the mutability rarely exceeded 10 percent.

A relation was found to exist between the mutability of the parent culture and the mutability of the offspring. Offspring of a highly mutable culture are likely to give more highly mutable cultures than the offspring of a less mutable one (table 11). The increase in the mutability, however, does not go behind a certain limit which is between three and five percent.

TABLE 11

Comparison between the mutability of parents and offspring.

MUTABILITY OF PARENT CULTURE PERCENT	NUMBER OF CULTURES	DISTRIBUTION OF MUTABILITY IN OFFSPRING										TOTAL
		0		1 Percent		1-5 Percent		5-10 Percent		10 Percent		
		Number of cultures	Percent	Number of cultures	Percent	Number of cultures	Percent	Number of cultures	Percent	No. of cultures	Percent	No. of cultures
0	17	88	77.9	5	4.4	13	11.5	5	4.4	2	1.8	113
less than 1	16	72	64.3	15	13.4	17	15.2	7	6.3	1	0.8	112
1–5	32	65	37.6	11	6.4	62	35.8	29	16.8	6	3.4	173
5–10	19	10	11.8	7	8.2	39	45.9	22	25.9	7	8.2	85
more than 10	7	5	25.0	1	5.0	9	45.0	3	15.0	2	10.0	20

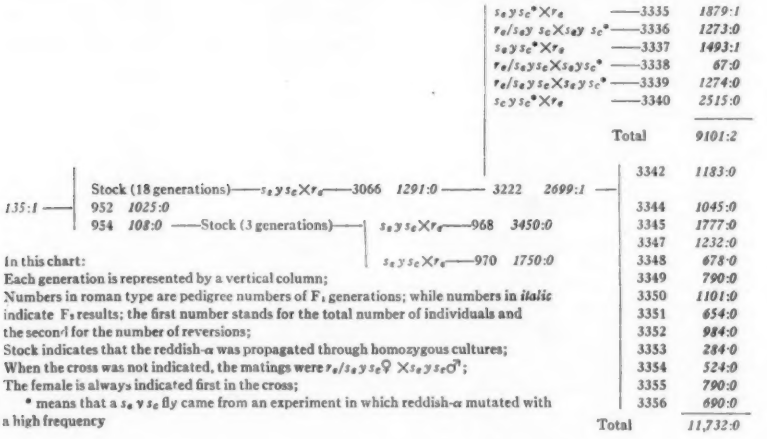
Constancy of reddish-alpha

As can be seen from table 11 constant cultures (with no reversions) were found among the offspring of highly as well as among the offspring of little mutable cultures. This was almost without exception. The lack of reversions was probably due in some of the cultures to the small numbers of individuals; in others, however, it was probably caused by the constancy of reddish-α.

Two males were taken from a very mutable culture which gave 16 reversions out of 97 individuals. They were mated to sepia yellow scute females from stock. In the F_2 generation from one of the matings, among 1307 flies, there was only one reversion; and in the F_2 from the other mating there were no reversions among 425 flies. Both of the males

came from a highly mutable culture; but offspring of one of them had very low mutability, and the offspring of the other was either of very low mutability or possibly constant. The number of individuals in this experiment was large enough to indicate a difference in mutability between the parents and the offspring.

It is evident from the chart that reddish-α can become constant or nearly so. Three lines originated from a culture which had very low mutability and which gave one reversion among 135 individuals. One of these lines was discontinued after one F_2 generation had been obtained in which no reversion was found among 1025 individuals. The other line


$s_e y s_c^+ \times r_e$ —3335 1879:1
$r_e/s_e y \; s_c \times s_e y \; s_c^+$ —3336 1273:0
$s_e y s_c^+ \times r_e$ —3337 1493:1
$r_e/s_e y s_c \times s_e y s_c^+$ —3338 67:0
$r_e/s_e y s_c \times s_e y s_c^+$ —3339 1274:0
$s_c y s_c^+ \times r_e$ —3340 2515:0
Total 9101:2
Stock (18 generations)—$s_e y s_c \times r_e$—3066 1291:0 — 3222 2699:1
135:1
952 1025:0
954 108:0 —Stock (3 generations)—$s_e y s_c \times r_e$—968 3450:0
$s_e y s_c \times r_e$—970 1750:0
3342 1183:0
3344 1045:0
3345 1777:0
3347 1232:0
3348 678:0
3349 790:0
3350 1101:0
3351 654:0
3352 984:0
3353 284:0
3354 524:0
3355 790:0
3356 690:0
Total 11,732:0
In this chart:
Each generation is represented by a vertical column;
Numbers in roman type are pedigree numbers of F_1 generations; while numbers in italic indicate F_2 results; the first number stands for the total number of individuals and the second for the number of reversions;
Stock indicates that the reddish-α was propagated through homozygous cultures;
When the cross was not indicated, the matings were $r_e/s_e y s_c ♀ \times s_e y s_c ♂$;
The female is always indicated first in the cross;
* means that a s_e y s_c fly came from an experiment in which reddish-α mutated with a high frequency

was continued for several generations, and 5308 offspring of females heterozygous for reddish-α were examined, none of them being a reversion. In these two lines reddish-α behaved as a constant gene in a total of 6333 individuals. The third line was kept as homozygous reddish-α for about eighteen generations. To test its mutability it was crossed to sepia yellow scute; and further propagated by backcrossing with sepia yellow scute males, and through crosses between reddish and sepia yellow scute flies. Among 24,823 flies of this line there were only three reversions. It might be questioned, however, whether these three reversions did not originate in a different way to the others, since all three were crossovers in the yellow-scute region. It is unlikely that they came into the experiment by contamination, since at that time no stock of the same genetic constitution was in existence in the laboratory. In any case, the frequency of

TABLE 12

Comparison of the frequency of reversions among the offspring of young and old females.

PEDIGREE NUMBER	NUMBER OF MATINGS	♀'s			♂'s										TOTAL ♀ AND ♂		
		Total	Reversions		0		1		2		Reversions		Total				
		r_c and y	+	s_c	r_c	$s_c y s_c$	$s_c r_c$	$y s_c$	$r_c s_c$	$s_c y$	+	s_c	r_c and y	Reversions	r_c and y	Reversions	Percentage of reversions
Offspring of young females																	
1283	10	453	21	4	205	182	2	6	2	2	22	3	399	25	852	50	5.9
1232	5	131	5	1	65	59	1	..	..	2	1	..	127	1	258	7	2.7
1285	1	9	1	..	13	1	..	..	..	..	1	..	14	1	23	2	8.7
1296	2	222	1	2	86	86	..	1	1	2	4	1	176	5	398	8	2.0
1299	2	84	5	2	47	48	..	..	..	..	3	1	95	4	179	11	6.1
1300	1	86	1	..	41	42	1	1	..	1	..	..	86	..	172	1	.6
1301	1	55	3	1	27	41	..	1	..	..	3	..	69	3	124	7	5.6
1547	2	138	5	..	43	62	4	1	1	1	6	1	112	7	250	12	4.8
1684	2	92	..	..	53	33	1	..	..	..	1	..	87	1	179	1	.6
1664	1	64	..	..	18	17	1	1	..	..	1	1	37	2	101	2	2.0
1727	1	19	1	1	12	9	..	..	..	..	2	1	21	3	40	5	12.5
1728	4	212	4	..	96	107	4	..	1	1	1	1	209	2	421	6	1.4
1730	1	82	2	..	37	38	..	1	..	..	1	..	76	1	158	3	1.9
1731	2	63	2	1	51	45	..	..	..	..	3	1	96	4	159	7	4.4
1732	5	268	7	1	97	139	..	2	3	..	4	..	236	4	504	12	2.4
1738	1	69	..	1	27	22	1	..	..	..	1	..	50	1	119	2	1.7
1607	1	75	1	..	39	43	1	1	..	..	1	..	84	1	159	2	1.3
1646	7	420	11	1	152	156	6	2	..	2	7	2	318	9	738	21	2.8
Total	49	2542	70	15	1109	1125	22	17	8	11	62	12	2292	74	4834	159	3.3

TABLE 12 (Continued)

Comparison of the frequency of reversions among the offspring of young and old females.

PEDIGREE NUMBER	NUMBER OF MATINGS	♀'s			♂'s										TOTAL ♀ AND ♂		
		Total	Reversions		0		1		2		Reversions		Total				
		r_c and y	+	s_c	r_c	$s_c y/s_c$	$s_c r_c$	y/s_c	$r_c s_c$	$s_c y$	+	s_c	r_c and y	Reversions	r_c and y	Reversions	Percentage of reversions
Offspring of old females																	
1283	10	468	2	..	198	212	8	4	1	1	3	..	424	3	892	5	.6
1232	5	268	..	..	109	79	3	2	..	..	..	..	193	..	461	..	..
1285	1	63	..	..	25	12	1	..	..	..	..	..	38	2	101	2	2.0
1296	2	113	1	..	45	50	..	1	..	..	1	1	96	2	209	3	1.4
1299	2	156	3	..	71	67	..	2	..	..	2	..	140	..	296	3	1.0
1300	1	62	..	..	25	21	1	1	..	..	..	..	48	..	110	..	..
1301	1	51	3	1	21	24	1	..	..	..	1	1	46	2	97	6	6.2
1547	2	114	3	..	53	50	1	1	..	2	4	..	107	4	221	7	3.2
1684	2	136	..	..	44	41	2	2	..	..	..	..	89	..	225	..	..
1664	1	50	..	..	14	19	1	..	..	..	1	..	34	1	84	1	1.2
1727	1	50	1	..	20	26	..	..	..	..	..	..	46	..	96	1	1.0
1728	4	166	..	..	89	65	1	1	1	2	1	..	159	1	325	1	.3
1730	1	48	1	..	24	24	..	..	..	..	..	..	48	..	96	1	1.0
1731	2	75	2	..	30	25	..	..	1	..	3	..	56	3	131	5	3.8
1732	5	256	3	..	123	125	..	1	4	..	1	1	253	2	509	5	.98
1738	1	17	..	..	10	8	..	..	..	..	..	..	18	..	35	..	..
1607	1	50	..	..	20	16	1	..	1	..	..	..	38	..	88	..	..
1646	7	322	2	1	145	159	2	2	2	4	9	1	314	10	636	13	2.0
Total	49	2465	21	2	1066	1023	22	17	10	9	26	4	2147	30	4612	53	1.1

reversions was so low as to be almost negligible; and reddish-α of that line behaved more like a constant than like a mutable character.

The evidence accumulated from the experiments with mutable miniature-α character of *Drosophila virilis* indicated that the mutablity of the miniature-α gene was affected by several other genes. It has been found that, in the presence of a certain dominant autosomal gene, an almost constant miniature-α becomes highly mutable. To determine if the absence of the proper modifying factor was responsible for the constancy of reddish-α, six crosses were made between reddish-α from the constant line and sepia yellow scute flies from the experiments in which reddish-α mutated with a high frequency. These crosses are marked with an asterisk on the chart. From the results it can be seen that the mutability of reddish -α was not influenced to any appreciable extent. Among a total of 9101 flies, only two reversions were obtained (both of which were crossovers in the yellow-scute region). This makes it probable that the constancy of reddish-α was due to the gene itself rather than to the absence of factors which would increase the mutability.

EFFECT OF THE AGE OF THE FEMALE ON THE FREQUENCY OF MUTATIONS

In the course of experiments with mutable reddish-α, it has been noticed that the proportion of reversions was higher among the first hatched flies than among the flies which hatched later. This difference in the proportion of reversions among the early and late hatched offspring could be accounted for in two ways. Either the reversions hatched sooner than the other classes, or the proportion of reversions was larger in the first laid eggs. To determine which of these two possibilities was more probable, a comparison was made between the offspring which came from the eggs laid by young females, and the offspring from the eggs laid by the same females when old. In several experiments, pairs were kept in one set of culture bottles until the first offspring began to hatch, and then they were transferred to new culture bottles. Offspring in the first bottles came from the eggs laid by the females younger than four weeks, and the offspring in the second bottles came from females older than four weeks. The summary of the data of eighteen experiments involving 49 females is given in table 12, from which it can be seen that, in all except one experiment, the percentage of reversion was larger among the offspring of young females. When the data of all the experiments are added, the percentage of reversions in the offspring of young females is three times the percentage of reversion in the offspring of old females.

The result of this experiment indicates that the age of the flies influences the frequency of mutations of reddish-α to wild-type. In old females, that frequency is appreciably lower than in the young females.

CROSSING OVER AND REVERSIONS

As has been already pointed out in one of the previous chapters, crossing over can not be responsible for the appearance of reversions in crosses with reddish-α. The behavior of reddish can not be compared with the behavior of bar in *Drosophila melanogaster*; where, as has been shown by STURTEVANT (1925), the appearance of unexpected wild-type flies was always connected with crossing over in the bar region. In the case of reddish-α (tables 2, 3, 12 and 13) about 87 percent of reversions are non-crossovers in the regions adjoining reddish.

There is, however, a connection between crossing over and reversions of reddish-α. An examination of the data given in table 13 reveals a marked increase of yellow-scute crossovers in reversion classes as com-

TABLE 13

Male offspring of s_e y s_c v/r_α females showing the amount of crossing over in different regions.

0	r_α $s_\alpha y s_c v$	7589 7230
1	$r_\alpha s_\alpha$ $y s_c v$	195 146
2	$r_\alpha s_\alpha v$ $s_\alpha y$	50 88
3	$r_\alpha v$ $s_\alpha y s_c$	1790 1785
1–3	$s_\alpha r_\alpha v$ $y s_c$	.. 5
2–3	$r_\alpha s_\alpha$ $s_\alpha y v$	2 3
Reversions	+ v $s_c v$	255 48 46
Total		19,232

pared with the same crossovers of normal classes. The values given in table 13 are directly comparable, since all the data were obtained from

the same females. Unequal distribution of crossovers is still more evident from the analysis given in table 14, where the data for reddish and reversion classes of table 13 are brought together. From table 14 it can be seen

TABLE 14

Comparison between crossovers occurring in reddish and reversion classes.

CROSSOVER REGIONS	CLASSES		PERCENT OF REVERSIONS
	Reddish	Reversions	
0	7589	255	3.3
$s_e - r_e$	195	0	0.0
$r_e - s_c$	52	46	46.9
$r_e - v$	1790	48	2.6
Totals	9626	349	3.5

that among the crossovers in reddish-scute region in 46.9 percent of possible cases reddish-α reverted to the wild type. On the other hand, in non-crossover class reddish-α reverted in 3.3 percent of possible cases, in the sepia-yellow crossover class there were no reversions and in the scute-vermilion crossover class the reddish-α reverted in 2.6 percent of possible cases.

From table 14 it could be seen also, that among 9626 reddish-α flies there were only 52 (0.54 percent) crossovers in reddish-scute region, while among 349 reversions there were 46 (13.2 percent)crossovers in the same region. The percentage of yellow-scute crossovers in reddish classes is lower than it would be normally expected. That deficiency, however, was probably caused by a poor viability of scute flies. An examination of the data given in table 13 shows that the scute class was in all except one case lower than its reciprocal class. A comparison of crossover percentages reveals that the crossing over in reddish-scute region was 24.4 times as frequent among reversions as among the normal classes.

The data presented above indicate that almost one half of the crossovers in reddish-scute region were reversions, or, when examined from a different angle, they show that the crossing over in reddish-scute region was 24.4 times as high among the reversions as among the normal classes. The observed facts may be accounted for in two ways: (a) by the assumption that the total amount of crossing over in reddish-scute region was not influenced by the occurrence of reversions but that the reversions occur about twelve times as frequently when the crossing over in reddish-scute region occurs than when there is no crossing over, or (b) by the assumption that whenever the reversion occurs in one out of about thirteen cases

crossing over in reddish-scute region will occur also, irrespective of whether this crossing over would have occured otherwise. According to the first assumption, it would be expected that the increase in the frequency of reversions would not change the total amount of crossovers in the reddish-scute region, but would only affect their distribution between the reversions and the normal classes. According to the second assumption, an increase in the amount of reversions would also result in the increase in the total of reddish-scute crossovers. According to either of the assumptions, however, the occurrence of reversions is stimulated with the close association of homologous chromosomes in reddish-scute region at the time when crossing over occurs.

In table 15 the data are summarized according to the amount of rever-

TABLE 15

Comparison between the frequency of reversions and the percentage of crossing over in the $r_e - s_c$ region

FREQUENCY OF REVERSIONS IN DIFFERENT EXPERIMENTS PERCENT	AVERAGE FREQUENCY PERCENT	TOTAL NUMBER OF FLIES	PERCENT OF CROSSOVERS IN $r_e - s_c$ REGION		
			Reversions	r_e and y classes	r_e and y and reversions
less than 0.5	0.3	4594	13.3	0.6	0.7
.5–1.0	0.8	3109	15.4	0.7	0.9
1–2	1.6	1348	9.5	0.5	0.6
2–3	2.3	2436	10.7	1.1	1.3
3–4	3.2	1455	6.5	1.1	1.2
more than 4	5.2	1625	16.5	1.1	1.8

sions obtained in different experiments. The results indicate that the amount of crossing over among reversions was approximately the same in the experiments which had a low frequency of reversions as in those in which the frequency was high. On the other hand, there is an indication that in the experiments which had a high frequency of reversions there was an increase in the total crossing over as well as in the percentage of crossing over in the reddish and yellow classes. These findings favor the second assumption.

It is unfortunate that it has not been possible to increase the amount of reversions much above five percent. The low frequency of reversions makes the reversion classes small and so prevents a more detailed analysis of the relation between the occurrence of reversions and crossing over in the reddish-scute region.

DISCUSSION

From the evidence obtained in different experiments with reddish-α the most fitting explanation for the unusual behavior of that character

seems to be the hypothesis that the gene for reddish-α reverts frequently to the gene for the wild-type. Several other hypotheses, which could explain the origin of wild-type flies in crosses with reddish-α, fail entirely to stand the crucial tests.

Any hypothesis explaining the behavior of reddish-α would have to assume that reddish-α is a sex-linked character and also an allelomorph of yellow. There can not be any question as to the validity of the first assumption. In the numerous matings in which reddish was used it never failed to show the characteristic behavior of a sex-linked character of Drosophila. The assumption of allelomorphism to yellow is well supported by: (a) linkage tests, which indicate that reddish-α is located in approximately the same region as yellow; (b) F_1 female progenies from crosses between reddish-α and yellow, which are always yellow; (c) F_2 results from these crosses, where only reddish-α and yellow males were obtained if the reddish-α parent used in the cross was from a constant line; and (d) by the fact that the reddish-α and yellow affect the same character, as it is the case with all known allelomorphs.

When reddish-α of a "mutable" line is crossed with yellow, all F_1 females are yellow, as expected; but among F_2 progenies, in addition to yellow and reddish-α flies, a few wild-type individuals appear. The number of wild-type flies varies in different cultures from zero to 20 percent. It is usually low, and rarely reaches above 10 percent. What is the origin of these unexpected wild-type flies? In addition to the hypothesis that they are due to the change of the gene for reddish-α to the gene for wild-type, is possible that they might be a product of: (a) the variablity of reddish-α; (b) recombination between reddish-α and yellow; (c) the complex nature of the reddish-α character; or (d) the abnormal behavior of chromosomes. Let us consider these possibilities in detail.

(a) If the exceptional wild-type flies were due to the variability of reddish-α, it would be expected that they would be genetically reddish-α. That, however, was not the case. A number of these flies was tested for two generations and they gave only wild-type progenies, without any reddish-α flies.

(b) It has been shown by STURTEVANT (1925) that in exceptional cases unequal crossing over occurs in the bar-region of sex chromosome of *Drosophila melanogaster*, and results in the recombination of allelomorphs. Similar process occuring in the reddish-α region could produce the wild-type flies. In that case, however, it would be expected that all wild-type flies would be crossovers. Quite the opposite was found, since only about

13 percent of the exceptions were yellow-scute crossovers, and almost none were crossovers in the sepia-yellow region. These results eliminate the possibility of the wild-type flies originating by unequal crossing over.

(c) By assuming that the reddish-α character is determined by duplicate genes a scheme could be devised which would explain the appearance of wild-type flies. The abnormally small and variable numbers of the wild-type flies could probably be explained by the assumption of differential viability. There are, however, other and more serious obstacles to that hypothesis. If the reddish-α character were determined by duplicate genes, one of them would be undoubtedly sex-linked and an allelomorph of yellow, and the other or others might be either autosomal or sex-linked. If any of the genes needed for the expression of the reddish-α character were an autosomal recessive, reddish-α would not behave as a sex-linked character. If it is assumed, however, that the autosomal gene is a dominant one, it would be expected to segregate freely with the gene in the sex chromosome. The expected result would be that the wild-type flies would appear among the progeny of heterozygous males as well as among the progeny of heterozygous females. That was not the case. Males from the cross reddish-α by yellow produced no wild-type flies, while the females from the same cross gave wild-type flies.

There are several reasons which eliminate the possibility of reddish-α being determined by duplicate genes located in the sex chromosome. It might be enough to consider one of them. If it is assumed that reddish-α is determined by two sex-linked genes, one of which is an allelomorph of yellow, then the wild-type flies observed in crosses between reddish-α and yellow, would constitute half of the crossovers between the duplicate genes determining reddish-α. It has been shown in table 14 that, in an experiment carried through sixteen generations, the average frequency of wild-type flies was about three percent. That would indicate that the other duplicate gene of reddish-α would have to be located about six units from yellow. If the wild-type flies were crossovers between the two duplicate genes it would be expected that all of them would be crossovers in the region six units on either side of yellow. The data given in table 10 show that they are not crossovers in the yellow-vermilion region, and also indicate that it is very unlikely that they are crossovers in the region on the other side of yellow.

(d) In all experiments, reddish-α showed a strictly sex-linked inheritance that eliminates the possibility of the unexpected behavior of reddish-α being due to an abnormal action of the autosomes. In numerous experiments made with reddish-α there was nothing to support the as-

sumption that the abnormal behavior of a whole sex-chromosome was responsible for the origin of the wild-type flies obtained in crosses with reddish-α. In table 16, the data are given which show that the wild-type

TABLE 16

Summary of experiments in which different regions of the original reddish-α chromosome were represented. (Solid line indicates the left-end of the original reddish-α chromosome and the broken line the replaced regions.)

TYPE OF THE X-CHROMOSOME	NUMBER OF EXPERIMENTS	NUMBER OF FLIES	
		Total	Reversions
	66	37,462	684
	12	4,549	84
	2	1,459	19
	33	15,686	223
	11	5,580	51
	9	4,214	35

flies occured in the experiments in which the whole original chromosome carrying reddish-α was present as well as in the crosses in which different parts of that chromosome were replaced by the parts from the other sex chromosome. From table 16 it could be seen that the small part of reddish-α chromosome located between sepia and scute (about 3 units in length) was sufficient to produce the abnormality; and the data discussed on page 384 indicate that a gene, rather than a region of the chromosome, is responsible for the origin of the wild-type flies.

Reddish-α was found to mutate in one direction only, namely, from reddish-α to wild-type. Tests involving a large number of progenies of reverted reddish-α failed to detect any mutation in the opposite direction. Moreover, the mutability of reddish-α was found to be limited to a very short period of development, which has been located at the maturation divisions of heterozygous females. No mutation was observed in somatic cells, in homozygous females, or in males. What could be the reason for that narrowly restricted mutability of the reddish-α gene? Experimental evidence does not give as yet any answer to this question. It may be possible that the restriction is due to the quality of the gene itself, or it may be also possible that the reddish-α gene is potentially mutable in other periods of development, but that the stimulus which produces the

immediate change is lacking except at the maturation divisions of heterozygous females. It has been found that the potentially mutable gene of miniature-α behaves almost as a constant one until it is stimulated to mutate by some other gene. Two such genes were isolated; both autosomal dominants. One of them induces miniature-α to mutate with high frequency in somatic cells, but does not affect the mutability of germ-cells. The other one induces a high mutability of the germ-cells, not changing appreciably the mutability of somatic cells. Some condition similar to that found in the case of miniature-α would account for the restricted mutability of reddish-α. The attempt, however, to induce somatic mutability in reddish-α by the gene which stimulates miniature-α to mutate somatically, was not successful.

Restriction of the mutability of a mutable gene to limited periods in the development of the organism is not an unusual condition. IKENO (1923) described two recessive characters in Plantago, which when selfed, gave regularly a small number of the wild-type individuals. These wild-type plants were interpreted as due to reversions occurring at the formation of gametes, or in the somatic cells closely preceding the reduction division. Mosaic plants, which would be due to mutations during the late somatic development, were observed very rarely, if at all. Similarly IMAI (1925a) found that the cream-colored flower character of Pharbitis when selfed gave regularly about 6 percent dark colored plants. Variegated flowers were rarely observed. In a line of Antirrhinum, BAUR (1926) obtained regularly about 2 percent crispa plants. Crispa is a dominant character and lethal when homozygous. The origin of crispa plants could be interpreted as being due to the mutability of the gene for the wild-type to the gene for crispa. Since no mosaic plants were observed the mutability in this case was probably limited to maturation divisions. IMAI (1925b) described a mutable character in Pharbitis which had the gametic frequency of mutations of 0.40 percent, and the somatic frequency of 0.17 percent. In Delphinium (DEMEREC 1927b) a character is known which mutates with high frequency in the early stages of the development of plants, and with very high frequency in the late stages of development, but does not mutate at all or mutates with a very low frequency in the intermediate stages. The difference between the mutability of reddish-α and the mutability of the genes just mentioned appears to be only in the fact that the period of the mutability of reddish-α is still more limited than the period of mutability of these genes. Consequently the difference between the mutability of reddish-α and such genes producing variegation in plants would be that these genes mutate at all stages of the develop-

ment, while reddish-α mutates at the maturation divisions of heterozygous females only.

An attempt has been made (CHITTENDEN 1927) to explain the behavior of variegated pericarp of maize and other similar variegations found in plants as due to the plastid inheritance. The reddish-α case, however, is distinctly chromosomal in inheritance and any hypothesis to explain the observed behavior as being determined by cytoplasm seems to be inconceivable.

SUMMARY

Reddish-α was found in two sister matings in the backcross involving the character branched as well as concave approximated and telescoped. In one of the matings, about one half of the males were reddish; and in the other only one reddish male was found. The possibility is not excluded, but the probability is low, that the single male came as a contamination from the culture which had many reddish males.

Reddish-1 was found (by Miss M. MOSES) seventeen months later in a mating involving the same characters as the one in which reddish-α was found. The branched used in the second case had a different origin from the branched used in the first case, but the concave approximated telescoped flies came from the same line.

To test the possibility that in the concave approximated telescoped line mutations to reddish occur frequently; 16,980 offspring of the backcrosses involving branched, approximated and telescoped characters were examined. No reddish flies were found.

Both reddish-α and reddish-1 are sex-linked and allelomorphs of yellow.

In F_2 generations from crosses between reddish-α and yellow, in addition to reddish-α and yellow flies, few wild-type ones appear. Experiments indicate that these wild-type flies are not due to crossing over between reddish-α and yellow, that they are not due to the abnormal behavior of chromosomes, nor to the complex genetic nature of reddish-α. They are interpreted as being reversions of the gene for reddish-α to the gene for wild-type.

The wild-type flies which originated as reversions from reddish-α are genetically constant.

Reddish-1 does not revert to wild-type.

Reddish-α reverts to wild-type only in females which are heterozygous for reddish-α and one of its allelomorphs, namely, wild-type, yellow or reddish-1. No reversions were observed in somatic-cells, in homozygous females, or in the males.

The frequency of reversions was found to be very variable. It decreased from 12.4 percent in the second generation after the origin of the reddish-α, to zero in the seventh generation.

By using for matings flies from cultures which gave the highest number of reversions it has been possible to keep the frequency of reversions for sixteen generations at about three percent.

A positive relation was found to exist between the mutability of the parent culture and the mutability of the offspring.

A line was isolated in which reddish-α behaved as an almost constant character.

The age of the female reduces the frequency of reversions.

It was found that, in the classes which originated by reversions, the crossing over in the yellow-scute region was increased 24 times over the normal amount. An analysis of the data indicates that the force which influences the reddish-α to revert to the wild-type at the same time causes the increase in the crossing over in the yellow-scute region.

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TWO NEW MUTANT CHARACTERS ON THE SPERMATHECAE OF THE FEMALES OF *DROSOPHILA MELANOGASTER*. CELL-DEGENERATION AND SUPERNUMERARY SPERMATHECAE.

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INTRODUCTION

In this work two mutant characters have been studied, both affecting the normal development of the spermathecae in the females of *Drosophila melanogaster*. The first character is a hereditary degeneration of the cells of the epithelium of the spermathecae. It was found in January 1921 by Doctor J. L. COLLINS as a mutation in a stock of curved flies which was extracted from a cross, black purple curved×jaunty. The second is the occurrence of the supernumerary spermathecae, a stock being produced in which the females all showed 3 instead of the normal number 2 spermathecae. This mutant was found in the stock which carried the hereditary cell-degeneration. The present investigation was carried out in the Genetics Laboratory of the UNIVERSITY OF CALIFORNIA during the fall semester of 1926 and the spring semester of 1927. I take great pleasure in thanking Professor E. B. BABCOCK for the facilities given me in the Genetics Laboratory and Doctor J. L. COLLINS for the material and for help and suggestions during the course of the work. Acknowledgment is also given to the INTERNATIONAL EDUCATION BOARD for the fellowship granted to me.

THE HEREDITARY CELL-DEGENERATION

Description

As far as it has been possible to observe the cell-degeneration is strictly limited to the epithelium of the spermathecae. The gross appearance is

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1, 2 or 3 black spots on the dorsal side of the posterior part of the abdomen (figure 1). When highly developed it can be seen with the naked eye; if the degeneration is slight, however, the spermathecae are hard to see, even with large magnification and a dissection of the fly is often necessary

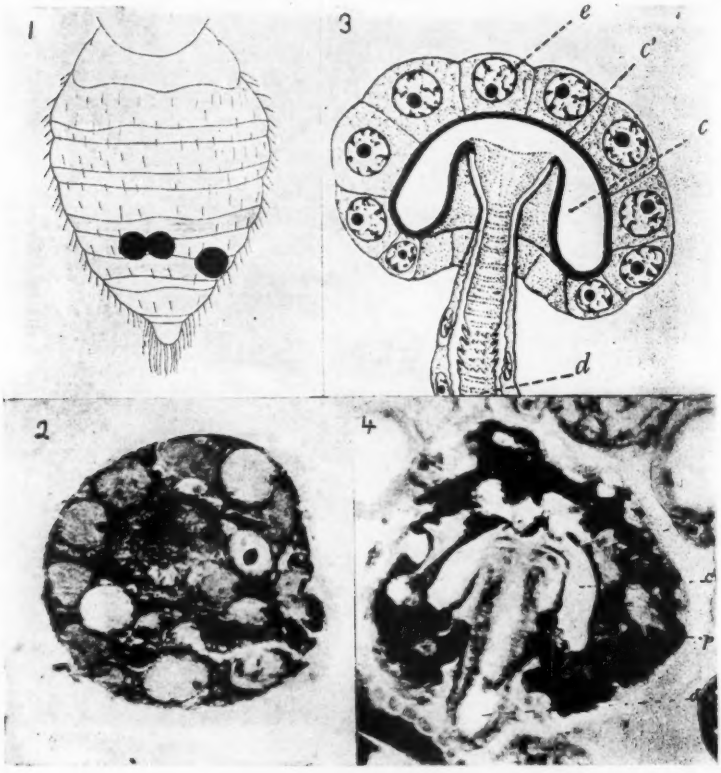

FIGURE 1.—Abdomen of female carrying 3 spermathecae each with a cover of degeneration tissue.

FIGURE 2.—Cross-section of the epithelial layer of a spermatheca at an early stage of degeneration. No nuclei are found in the epithelial cells which are filled with a brown pigment. Microphotograph Magnification ×800 diam. 5μ. Haematoxylin.

FIGURE 3.—Longitudinal section of a normal spermatheca; c=cavity; c^1=cuticle; d=duct; e=epithelium. Photograph after drawing of NONIDEZ (1920, p. 215).

FIGURE 4.—Longitudinal section of a highly degenerated spermatheca. Brown pigment covers most of the epithelium in which all cellular structure is lost. Microphotograph Magnification ×800 diam. 5μ. Haematoxylin. c=cavity; d=duct; e=epithelium; p=pigment.

to make a safe decision. The spermathecae are 2 (or 3) mushroom-shaped bodies connected with the uterus by narrow ducts and serving as storage organs for the sperm. Their terminal cavity is surrounded by a brown cuticle, secreted by the epithelium forming the walls of the organ (figure 3).

As the degeneration proceeds there is a deposition of dark brown pigment in the epithelial cells. Figure 2 shows a cross-section of the epithelial layer at an early stage of pigment formation. The cell structure is still retained, but the nuclei are gone and the cells are filled with a brown pigment. Later on the cell structure is completely gone and the brown pigment forms a complete bag around the spermathecae. A section through such a spermatheca is shown in figure 4.

The deposition of pigment seems often to be checked at an early stage and the gross appearance is then a few brown granules scattered throughout the epithelial layer, a type which I have called d_g-*a* for sake of convenience as contrasted with d_g-*b* in which the pigment forms a complete bag around the spermathecae, and is visible as conspicuous black spots such as shown in figure 1. There seem to have been present in the stock modifiers, reducing the pigment deposition, for by selection, the percentage of d_g-*b* females was considerably raised.

However, even after 15 generations of selective inbreeding both types occur, showing that they may appear as modifications of the same genotype. The males of the d_g stock are normal as far as has been observed, but no dissection of males has been made. The data given below on development and inheritance are therefore concerned with the females only.

Development and variability

The degeneration has never been observed in larvae or pupae and indeed not in newly hatched flies. Observations were made on 42 virgin females which were isolated at the pupal stage (throwing out the males as soon as possible after emergence). The females were examined as often as possible during the first 72 hours; visible pigment did not develop until 24 hours after emergence and developed in some cases as late as 72 hours after emergence. Classification of d_g *versus* non-d_g flies can therefore be safely made only on females at least 3 days old; a circumstance which complicates the genetic analysis considerably. By counting the flies twice a day, for instance, one could run pure cultures of the mutant stock, finding only a single individual with degenerated spermathecae, now and then. If only sufficiently-old females are examined, the degeneration frequency in pure cultures is now 100 percent.

There is still variation in the degree of degeneration and a few experiments were carried out to determine the influence of external factors. Breeding at 24° and 31° C did not give any difference in the degree of degeneration, nor did a variation in the moisture of the cultures have any effect. To test the effect of the amount of food, vials were made up with 5cc banana-agar food in each and in these were placed 50, 100, 150 or 200 eggs from cultures of the mutant stock. The results are given in table 1 under 2 groups (1) 50-100 eggs—(2) 150-200 eggs. The degree of cell-degeneration is expressed by the relative proportion of d_g-*a* and d_g-*b* flies.

TABLE 1

Effect of amount of food upon cell-degeneration.

NUMBER OF BOTTLES	NUMBER OF EGGS PER BOTTLE	AVERAGE NUMBER OF FLIES	d_g-a	d_g-b
12	50–100	18.42	44	52
6	150–200	42.80	38	105

Although the numbers are small, there is a clear indication of increasing degeneration with decreasing amount of food.

It is not likely that the degeneration is due to infection or some other external cause as it segregates out in Mendelian ratios. The sex-ratio in the mutant stock is normal, which shows that the females with the abnormal spermathecae are equal in viability to the males which are normal. The viability of the d_g classes in relation to the non-d_g classes in segregating populations is discussed under the treatment of the results from the backcrosses.

The fertility of the females is good. It has not been possible to ascertain whether the sperm can enter an abnormal spermatheca, and whether such a spermatheca can again give off sperm for fertilization, but this seems likely in view of the good fertility of the females. It was thought that fertilization was perhaps necessary for the cell-degeneration, as the development does not begin till after the time when the females have usually been fertilized in mixed cultures. The foregoing experiment with isolation of virgin females showed that the cells degenerate in virgin females as well.

Inheritance

The character is recessive: when d_g females are outcrossed to males of other stocks the F_1 females have never shown cell-degeneration. At the time the character was found some work was done with it and a stock had been built up which contained besides d_g the 2 second-chromosome mutants, curved wings (*c*, locus 75.5) (BRIDGES AND STURTEVANT

1914) and cinnabar eyes (c_n, locus 57.5) (CLAUSEN 1924). This work had shown that the mutant was probably due to a factor in the second chromosome. To get some data on this according to the standard Star Dichaete method, a c_n d_g c female was outcrossed to a Star Dichaete (*SD*) male and F_1 *SD* males backcrossed to females of the c_n d_g c stock. It was found, however, that Star in this cross was constantly overlapping the normal type; the characteristic roughness of Star eyes was often unseen but, as was found later, the Star flies could be picked out by the smaller size of the eyes. The first backcross gave $28 d_g$: 216 non-d_g. It was suspected, however, that some of the flies classified as non-d_g were genetically d_g, which later experiments proved to be true. The low percentage of d_g flies was probably due to the fact that classification had been made on too young individuals.

After selection for extreme type of degeneration had been carried on for some generations, females and males from the d_g stock were outcrossed to wild males and females, and F_1 individuals from each kind of cross were mated together in 2 mass cultures and 2 single pair cultures. The results of the F_2 are given in table 2.

TABLE 2

Female offspring from the mating of F_1 individuals heterozygous for c_n d_g c.

CULTURE NUMBER	+++	$c_n d_g c$	$+ d_g c$	c_n ++	++ c	$c_n + c$
35	65	14	1	9		3
36	39	7	3			1
Total	104	21	4	9		4
F_2 mass	155	50	3	5	4	13
Total F_2	259	71	7	14	4	17

In this, as in the following tables the symbols standing for the various mutant genes and + for their corresponding wild-type allelomorphs are used to designate the phenotype of the classes. Thus, +++ is used to designate individuals which are normal as regards the 3 mutant characters, cinnabar eyes, d_g and curved wings; the c_n d_g c class comprises the individuals which show all 3 mutant characters; whereas the $+ d_g$ c class includes the individuals which show d_g and curved wings, but not cinnabar eyes. The ratio of non-d_g:d_g flies in the F_2 is 270:102. On the basis of one factor difference the expected numbers would be 279:93; so the results agree with the assumption that cell-degeneration depends on a single recessive factor. It is clear that this factor is carried both by males and females although it expresses itself phenotypically only in the latter. As

to the location of this factor, the results show that it is located in the second chromosome, somewhere near curved. To determine the locus of d_g females heterozygous for c_n d_g c were backcrossed to males homozygous for c_n d_g c.

The results from this cross are listed in table 3.

TABLE 3

Female offspring from backcrossing females heterozygous for c_n d_g c to c_n d_g c males.

CULTURE NUMBER	+++	$c_n d_g c$	$+d_g c$	c_n++	$c_n d_g$+	++c	c_n+c	+d_g+	TOTAL
52	52	12	1	12					77
53	63	34	2	4			2		105
54	24	20	1	3			3		51
55	13	7		1					21
56	63	34	6	8					111
58	22	14	2	7			3		48
59	22	12	4	3			1	2	44
60	34	28	3	8		1		1	75
61	39	20	5	3	2		1		70
88	48	34	5	7		1	7		102
89	54	30	3	4		1			92
90	61	71	11	8		1		1	153
91	112	135	18	16			1		282
92	59	62	9	4		1	1		136
Total	666	513	70	88	2	5	19	4	1367

From the results it is clear that the factor d_g is very near to c. In the class c_n+c there is an excess of individuals as compared with the corresponding crossover-class $+d_g+$. This is probably due to the fact that some of these individuals are really c_n d_g c in which the abnormality has failed to develop. If we compute the crossover value d_g-c only on the basis of the crossover-classes showing degenerated spermathecae, we find a value of 1.0 percent. The order of the genes and the relation between single and double crossing over will be discussed later, taking into account data from all the backcrosses.

There is in this cross a deficiency of 23 percent in the class c_n d_g c which may be due to a lower viability of the triple mutant type as compared with the wild-type. To get correct crossover values in cases with a notably lowered viability, one has to make *balanced viability crosses*, that is, different combinations of factors are used in crossing. Besides the cross in table 3 one further backcross was made: c_n++/+d_g c ♀ ♀ × c_n d_g c♂ ♂. To obtain females of this constitution c_n d_g c females were outcrossed to wild males and an F_2 was raised. F_2 d_g c individuals were mated together

and F_2 d_g c females were mated to males of cinnabar stock. Wild-type females from this cross, with the constitution $c_n++/+d_g\ c$ were mated to $c_n\ d_g\ c$ males. The results from these matings are given in table 4.

In this cross there is no deficiency of the d_g classes in relation to the non-d_g classes; the same holds true for the F_2 (table 2) and there is no

TABLE 4

Female offspring from backcrossing $c_n++.+d_g\ c$ females to $c_n\ d_g\ c$ males.

CULTURE NUMBER	$+d_g c$	c_n++	$c_n d_g c$	$+++$	c_n+c	$+d_g+$	$c_n d_g+$	$++c$	TOTAL
81	23	19	5		2			1	50
82	26	31	4	4			1		66
83	17	26	6	4	2			2	57
84	48	35	7	3					93
85	32	23	5	2			1		63
86	13	9	1	3	1				27
87	30	25	7	4	1				67
Total	189	168	35	20	6		2	3	423

significant deviation in another later backcross (table 5). It is probable, therefore, that the large deficiency in the first backcross (table 3) is not due wholly to lower viability of the d_g class, but is rather due to the fact that the females had to be kept for many days, and it was noted that there was sometimes a heavy loss of females during this time. In the later experiments this was avoided through a better method of keeping the females. The general impression is that the d_g stock is of good viability, although the $c_n\ d_g\ c$ type is not equal to the wild-type, which is not an unexpected condition in any stock containing 3 mutant genes. The d_g flies in table 4 give a crossover value $d_g - c$ 0.9 percent.

The next step in the location of d_g was to find a gene nearer to c and d_g than c_n; such a gene is Lobe^2 (L^2 locus 72.0) a dominant factor which has the effect of reducing the eyes. A Lobe^2 male was mated to a $d_g\ c$ female and F_1 Lobe$_2$ females were mated to $d_g\ c$ males. The results of this backcross are given in table 5.

The crossover value between d_g and c, basing the calculation only on the d_g classes is 1.2 percent.

The data do not allow a safe decision as to whether the genic order is $c_n\ d_g\ c$ or $c_n\ c\ d_g$, though the evidence is slightly in favor of the order $c_n\ d_g\ c$. There seems to have been in the experiment a low percentage of crossing over between c_n and c, 11.9 and 15.5 as compared with the standard value 18.0. There is further a high percentage of double crossing over. Although the amount of crossing over is thus somewhat abnormal

it is safe to conclude that d_g lies within about one unit to the right or left of curved (75.5 ± 1.0).

TABLE 5

Female offspring from backcrossing L^2++/+d_g c females to d_g c males.

CULTURE NUMBER	L^2++	+d_gc	+++	$L^2 d_g$c	L^2+c	+d_g+	$L^2 d_g$+	++c	TOTAL
66	15	16					1		32
67	66	41	5					3	115
68	55	55	4					1	115
69	65	54	2	3	1			1	126
70	37	25	1		1		1	1	66
71	44	47	1	1		1		1	95
72	52	57	2	3	2			3	119
73	31	26	1	2	1	1			62
Total	365	321	16	9	5	2	2	10	730

TABLE 6

A summary of the 3 backcrosses, including only the d_g classes, on which basis the computations of the crossover percentages are made.

COMBINATIONS	−−	+−	−+	++	TOTAL	PERCENTAGE CROSSING OVER: c_n−d_g	d_g−c	c_n−c	double c_n−d_g−c	L^2−d_g	L^2+c	double L^2−d_g−c
c_n d_g c/+++	513	70	2	4	589	11.9	1.0	12.2	0.6			
c_n++/+d_g c	189	35	2	0	226	15.5	0.9	16.4	0.0			
L^2++/+d_gc	321	9	2	2	332		1.2		0.6	3.3	3.9	0.6

THE HEREDITARY BASIS FOR SUPERNUMERARY SPERMATHECAE

During the study of inheritance of the cell-degeneration it was necessary to dissect a large number of females. Some of the cultures were found to have many females with three spermathecae instead of two, which is the normal number.

The form and function of the spermathecae has been described by NONIDEZ (1920), while STURTEVANT (1926) has examined the intraspecific variability as to number of spermathecae. He examined specimens from wild stocks of *D. melanogaster*, *D. simulans*, F_1 hybrids between these two, *D. funebris*, *D. imigrans*, *D. buskii*, *D. repleta* and found two specimens with three spermathecae, all the others having two. The number of spermathecae thus seems to be a constant character and in the cases examined the species belonging to the same genus show the same number of spermathecae.

STURTEVANT (1926) describes a stock of *D. melanogaster* which showed a higher percentage of flies with three spermathecae and from which he

established a race in which from 25 to 75 percent of the females had three spermathecae. The genetic analysis has so far indicated that at least two factors are involved in the production of the extra spermatheca in this race.

In our d_g stock most of the individuals with three spermathecae had three of equal size; however, in some cases one was smaller than the other two. In individuals with two spermathecae one was sometimes larger than the other and often constricted. Occasional flies with four spermathecae were also found.

During the course of the investigation of d_g selection for three spermathecae was also carried on by always choosing flies from the cultures showing the highest percentage of three spermathecae. In a short time a stock was obtained which appeared to be homozygous for this new character. Two cultures counted at this time gave 205 with three spermathecae to 10 with two spermathecae. This stock was continued for several generations by brother-sister mating with no attention paid to the number of spermathecae. Then two cultures were again counted, giving 293 with three spermathecae to 4 with two spermathecae, which is 98.6 percent with three spermathecae.

The apparent ease with which a homozygous race was established indicated that the genetic basis for this character was perhaps simpler than in the case reported by STURTEVANT. It was, therefore, decided to attempt to determine the genetic basis for the inheritance of three spermathecae in this stock. The determination of the number of spermathecae was always made by dissecting the flies, a fact which in itself limits the number of flies which can be examined. Females from the three spermathecae (s_p^3) race were crossed with Star Dichaete (SD) and the F_1 SD ♂ ♂ were backcrossed to s_p^3 ♀ ♀, producing the following classes and numbers of female progeny.

$SD+$	SDs_p^3	$S++$	$S+s_p^3$	$+D+$	$+Ds_p^3$	$+++$	$++s_p^3$	total
81	—	78	1	45	2	66	32	305

The flies that have S or D or both do not develop s_p^3 (with the exception of 3 flies). This, therefore, indicates that there are in the second and third chromosomes recessive genes that contribute to the production of the third spermathecae. Since only a portion of the non-Star and non-Dichaete individuals show three spermathecae there must also be segregation in one other pair of chromosomes. If there were no other genes involved in the production of s_p^3 than those in the second and third chromosomes, then all the non-Star non-Dichaete flies should be s_p^3.

The segregation observed in the non-Star non-Dichaete flies cannot be due to a gene in the X-chromosome, because all the females of this culture have the X-chromosomes from the $s_p{}^3$ stock. Therefore, the segregation in non-Star non-Dichaete flies is probably due to a recessive gene in the IV-chromosome (table 7). A definite proof of the existence of such a gene in the fourth chromosome can only be delivered through linkage tests with a fourth chromosome mutant. This would require additional mutant stocks and additional time neither of which was available.

TABLE 7

Comparison of observed and calculated results on the basis that $s_p{}^3$ is due to the simultaneous action of three recessive genes, one in each of the II, III and IV chromosomes.

CHARACTER	*SD*	*S*	*D*	+	$s_p{}^3$	TOTAL
Observed numbers	81	78	45	66	35	305
Calculated numbers	*76*	*76*	*76*	*38*	*38*	*304*

The deviation from the 1:1 ratio in the Dichaete and normal classes cannot readily be explained, but it is probably due at least in part to difficulties in recognition of characters. The culture also involved curved, a wing character brought in by the d_g stock, which may have in some way interfered with classification. However, if we consider the segregation of $s_p{}^3$ and non-$s_p{}^3$ on a three chromosome (II, III and IV) basis the correspondence of observed and calculated numbers is very good.

Observed 270.0 non $s_p{}^3$ to 35.0 $s_p{}^3$
Calculated 266.7 non $s_p{}^3$ to 38.1 $s_p{}^3$

These results do not exclude the possibility of essential genes for $s_p{}^3$ being in the X-chromosomes; for these flies were all homozygous for these chromosomes. That the sex chromosomes are not involved, however, is indicated by the results of another backcross in which F_1 ♀ ♀ were used.

TABLE 8

Results of crossing ♀ ♀ of the constitution $\frac{+\ c_n\ d_g\ c\ s_p{}^3}{S++++}\ \frac{+s_p{}^3}{D+}$ *with* $\frac{+\ c_n\ d_g\ c\ s_p{}^3}{+\ c_n d_g\ c\ s_p{}^3}\ \frac{+s_p{}^3}{+s_p{}^3}\ \frac{s_p{}^3}{s_p{}^3}$ ♂♂.

Classes	*SD*	*S*	$c_n D$	$c_n c$	$c_n c s_p{}^3$	*D*	Sc_n	*Sc*	Total
Number	29	13	35	22	12	20	3	1	
Classes	$S\ c_n\ D$	+	c_n	c	$s_p{}^3$	$D\ s_p{}^3$	$c_n\ D\ s_p{}^3$	$SD\ s_p{}^3$	
Number	1	13	5	1	1	1	1	2	160

A ♀ from a cinnabar- d_g- curved- s_p^3 stock was crossed with a SD♂ and F_1 SD ♀ ♀ backcrossed to males of the c_n- d_g- c- s_p^3 race. Three backcross cultures gave the results in table 8. In this table curved-Dichaete flies are listed with the Dichaete.

If we consider the ratio of s_p^3 to non-s_p^3 we get, as before, evidence of a segregation in only 3 chromosomes.

Observed 143 non-s_p^3:17 s_p^3
Calculated 140 non-s_p^3:20 s_p^3

The male and female backcrosses thus give similar results showing that the sex chromosomes do not carry genes essential for the s_p^3 character. The conclusion is reached, therefore that the extra spermathecae in this race is dependent upon recessive genes in the second, third and fourth chromosomes. The appearance of Star s_p^3 and Dichaete s_p^3 flies may mean that in some cases the third spermathecae can be produced when either the second or third chromosome genes are in a heterozygous condition if the other two sets of genes are in a homozygous condition.

No definite evidence has been obtained relative to the linear location of any of the three s_p^3 genes. On the suggestion of DR. STURTEVANT females from cultures of SD from the stock used in this analysis were dissected to determine whether any of them carried 3 spermathecae. Two single pair cultures and one mass culture gave 73 SD:39 $S+$females all with 2 spermathecae; no $+D$ and $++$females. A probable explanation of this result is that a lethal has arisen by mutation in the second chromosome allelomorphic to the S gene, thus producing a balanced lethal stock breeding true for S. A culture, segregating for S, gave the result 12 SD:8 $S+$:7$+D$:1$++$females, all with 2 spermathecae. In all 140 females were examined, all had 2 spermathecae. It is not likely, therefore, that there are in the SD stock used in the analysis of the supernumerary spermathecae any genes which would interfere with the analysis.

As has been mentioned, the number of spermathecae, is a fairly constant character within a species, and as a rule uniform for all the species of a genus. It is, therefore, of interest to find such a character arising as a mutation in one species, giving rise to a race breeding true for a number of spermathecae, different from that which is characteristic of the Drosphilidae.

The genetic basis of this character provides an interesting side light on the problem of organic evolution. Against the theory of evolution through the selection of small independent mutations, it is urged as a main difficulty, that complex organs which must depend upon the interaction of many genes cannot be thought to arise through a series of quite indepen-

dent random mutations. So far all evidence indicates random mutation in *D. melanogaster*. It is reasonable to assume that it is the case here. Three mutations have occurred, each of which apparently has no influence upon development, but which together interact to cause the development of a complex organ, an extra spermatheca. This tends to show that the criticism of the theory, that mutations form the basis of organic evolution, may not have the importance often attached to it.

SUMMARY

1. In this work is studied a hereditary cell-degeneration on the spermathecae of the females of *Drosophila melanogaster*. It arose as a mutation in a stock of curved flies and has been proved to be dependent on one single recessive factor located in the second chromosome near curved 75.5 ± 1.0.

2. The abnormality appears only in adult females some time after emergence and consists in a deposition of a dark brown pigment in the epithelial layer of the spermathecae; in highly degenerated spermathecae the pigment forms a complete bag around the spermathecae, and is visible as conspicuous black spots on the abdomen of the females.

3. In pure stock nearly all the females show the cell-degeneration, but the degree of degeneration is variable and has been shown to be influenced by the amount of food in the cultures, such that with a smaller amount of food there is higher degree of degeneration.

4. The viability and fertility of the d_g females are good.

5. During the cell-degeneration investigations a stock was established which was homozygous for the new mutant character, supernumerary spermathecae, that is, 3 instead of the normal number 2.

6. A genetic analysis showed this character to be dependent upon recessive factors in the second, third and possibly fourth chromosomes.

7. It is pointed out that this character has an interesting bearing on the problem of organic evolution.

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A FURTHER STUDY OF THE SO-CALLED MUTATION AT THE BAR LOCUS OF DROSOPHILA

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INTRODUCTION

An earlier study (STURTEVANT 1925) of the "mutations" at the bar locus of *Drosophila melanogaster* led to the conclusion that these mutations are due to unequal crossing over at the bar locus. Reverted bar is no-bar, having no allelomorph at the bar locus; double-bar (the ultra-bar of ZELENY) has two bar genes lying next each other in the linear series of genes.

The present paper gives the results of a few experiments designed to clear up certain points left doubtful by the earlier study.

TIME OF OCCURRENCE OF BAR MUTATION

ZELENY (1921) recorded five cases in which more than one reversion appeared in a single culture, and concluded from this evidence that reversion probably occurs, sometimes at least, in oogonial cells. Since the five cultures in question were not from individual females they do not prove that several reversions may be produced by a single mother, but they are at least suggestive. If, following this suggestion, one assumed that unequal crossing over leading to bar reversion occurred at some stage earlier than the maturation divisions, it would be expected that normal crossing over between forked and fused would occur after it. The result should be that about 3 percent of the "mutations" at the bar locus would appear to be unaccompanied by forked-fused crossing over—and the data on record show a small number of such exceptional cases. A further consequence, open to experimental test, should be that bar reversion would not interfere with crossing over in neighboring regions.

[1] Contribution from the CARNEGIE INSTITUTION OF WASHINGTON.

Accordingly an experiment has been carried out with females homozygous for infrabar (used instead of bar in order to make the separation for eye-color easier), and heterozygous for garnet-2 forked and fused (see figures 1 and 2).

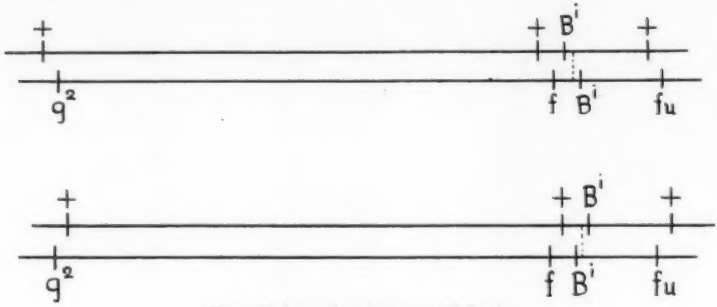

Figure 1 (above) and Figure 2 (below).

TABLE 1

Offspring from $\frac{B^i}{g^2 f\, B^i f_u}$ ♀ × $g^2 f\, B^i f_u$ ♂.

B^i									B^iB^i				+			
0		1		2		1,2		Total	♀*		♂		♀*		♂	
+	$g^2 f f_u$	$f f_u$	g^2	f_u	$g^2 f$	f	$g^2 f_u$		f_u	$g^2 f$	f_u	$g^2 f$	f_u	$g^2 f$	f_u	$g^2 f$
19536	14939	2351	2678	574	647	4	7	40736	4	2	2	6	4	7	11	4

* The "mutant" females were, of course, all heterozygous for B^i, received from the father.

A small series (1647 individuals) from $\frac{f\, B^i}{g^2 \quad B^i f_u}$ also gave a reversion ($f\, f_u$) that was not a $g^2 f$ crossover. In the two series there is a total of 41 "mutant" individuals, all of them crossovers between forked and fused, but none of them garnet-2 forked crossovers. There are 5040 garnet-2 forked crossovers among 40736 unmutated offspring of the table, or 12.4 percent, in good agreement with the 12.1 percent recorded by MORGAN, BRIDGES and STURTEVANT (1925).

If mutation and garnet-2 forked crossing over are unrelated, the chance that none of the 41 mutant individuals should be a garnet-2 forked crossover is therefore $(1-.124)^{41}=.004$, or 1 in 250. One may safely conclude that the unequal crossing over that gives rise to mutation at the bar locus

interferes with crossing over in the garnet-2 forked region, just as does ordinary crossing over between forked and fused, and hence that the unequal crossing over occurs at the same time as ordinary crossing over.

There must, therefore, be other explanations of the two relations that led to the experiment. The occurrence of mutations not accompanied by forked fused crossing over is open to two interpretations, as previously pointed out (STURTEVANT 1925). Crossing over between sister strands is a possible explanation, but if it occurred at all would be expected to give many more apparent non-crossover mutations than have been observed. The most probable view seems to be that all such records are due to experimental errors. It is to be noted that none occurred in the present series of experiments.

As pointed out above, ZELENY's data do not prove that more than one mutation was produced by a single mother in any case. However, in the present series there were four separate cultures in which a single mother produced two mutant offspring, as follows:

Culture	*Mutant offspring*
21794	$B^iB^if_u$, $g^2fB^iB^i$
22254	f_u, g^2f
23611	f_u, f_u
23898	$g^2fB^iB^i$, g^2f

Examination of these data shows that in three of the four cases the two individuals cannot have been produced by one crossing over.[1] In the first case, for example, the crossing over that produced a $B^iB^if_u$ chromosome would also give a g^2f (figure 1)—but could not give $g^2fB^iB^i$, the combination actually recovered. It follows that the production of more than one mutant offspring by a single mother must be due to the occurrence of more than one unequal crossing over in that mother. Whether or not such coincidences are more frequent than would be expected with a random distribution can hardly be determined without the collection of a vast amount of accurate data.

RELATIVE NUMBERS OF REVERSIONS AND DOUBLE-BARS

On the hypothesis of unequal crossing over a homozygous bar stock should produce equal numbers of double-bar and wild-types "mutants," but all observers have found distinctly more wild-type than double-bars.

[1] Unless by chance one of the rare "normal" forked fused crossovers followed the mutation in one egg and not in the other in each case. The chance that this should have happened in all three instances is negligible.

I have suggested (STURTEVANT 1925) that this is due to two factors—lower viability of double-bar and greater likelihood of overlooking mutant individuals. The viability factor can be directly measured. Using the inbred stocks prepared for the facet counts recorded in my earlier paper, I have made counts from double-bar over round mated to round and to bar. A small series gave 60 double-bar over bar females to 82 bar over round, and 126 double-bar males to 161 round males. Equality is expected in each case, and the deviations are due to differential viability. It follows that, in the combinations met with as mutant individuals in a homozygous bar stock, double-bar has a viability about 75 percent that of round.

ZELENY (1921) records the production of 52 reversions and 3 double-bars among 85008 specimens examined. In addition there were at least 5 double-bars that were not tested, so that we may take 52:8 = 6.5:1 as the observed ratio. ZELENY argues that there were probably very few mutant individuals overlooked—probably only a few of the double-bar females. I obtained (STURTEVANT 1925) 8 reversions and 2 double type from homozygous bar, 18 reversions and 3 double types from homozygous infrabar, and 2 reversions and 3 double types from bar over infrabar—a total of 28:8 = 3.5:1. This is somewhat closer to the expected equality than ZELENY's 6.5:1, but the experiments here reported for homozygous infrabar gave 27:14 = 1.9:1. This is still a definite excess of reversions, over what can be accounted for by differential viability, especially as the viability difference is less for double-infrabar than for double-bar, but more carefully conducted experiments and more experience having lowered the ratio from 3.5:1 to 1.9:1, I feel confident that there is still room for technical improvement sufficient to remove the remaining discrepancy.

POSITION EFFECT

It was shown (STURTEVANT 1925) that two bar genes lying in the same chromosome are more effective in reducing facet number than are the same two genes when they lie in different chromosomes. It seemed possible that such a relation might also hold for non-allelomorphic genes, but the one test, now to be described, has given a negative result.

The two dominat genes delta and hairless lie 3 units apart in the third chromosome (BRIDGES and MORGAN 1923). Hairless causes a reduction in bristle number, and delta partially counteracts this effect. These genes were selected for study because they lie close together, and because bristle number is convenient for quantitative work. An inbred strain was established, in which repeated brother-sister matings and repeated use of crossovers between delta and hairless made it probable that modifiers were

uniform. Matings were made in parallel, delta×hairless and delta hairless×wild-type. All offspring from these matings were reared at 26°C. The results are shown in table 2.

TABLE 2

Number of dorsocentrals.

Mating	3	4	5	Total	Mean
Δ X H	5	95	0	100	3.95
Δ H X +	10	167	1	178	3.95

While the numbers here are small, the exact agreement in mean number of dorsocentrals makes it seem certain that there is no position effect.

As pointed out in my earlier paper, the pairing of homologous chromosomes that occurs in somatic divisions in Drosophila suggests that there may be a position effect normally present of such a nature that allelomorphs reinforce each other's effects. Such an effect was offered as an explanation of the different dominance relations observed in triploids and in cases of translocation (MORGAN, BRIDGES and STURTEVANT 1925). A new set of facts makes this interpretation now seem improbable. I have shown (STURTEVANT 1926) that the C_{III} present in ebony and other stocks is due to the inversion of a large section of the third chromosome, and there is now evidence that the $C_{II\ L}$ of the curly stock and two other $C_{III\ R}$'s are due to similar inversions. In flies heterozygous for such inversions the position relations of many genes are greatly changed, but no difference in somatic appearance has yet been detected in such flies. It follows that changes in position of this order are not usually of developmental significance. One may conclude that the association of the two elements in double-bar is much more intimate than the association between identical loci in homologous chromosomes, and that the "position effect" perhaps rests on something more than mere closeness together.

FACET COUNTS OF DERIVED TYPES

Facet counts recorded (STURTEVANT 1925) for a reversion from bar and one from infrabar suggested that these derived rounds might be different from the wild-type, since both strains gave lower facet numbers with bar-infrabar (one of them also with double-bar and in males) than did wild-type. The inference that there is a wild-type allelomorph of bar, absent in reversions, and having an effect on facet-number opposite to that of bar, was further tested by studying an infrabar from bar-infrabar over round, and a bar from double-bar over round. Both of these derived types when tested against double-bar, gave somewhat larger facet-numbers than the controls (original infrabar and original bar, respectively). It was suggested

that perhaps the derived types carried wild-type allelomorphs, which partially counteracted the effects of the bar and infrabar genes. However, the differences were small in all these experiments, and it was emphasized that more tests were needed.

Several new derived types have now been studied, and have not borne out the assumption of a wild-type allelomorph.[2]

Two new infrabars, both from double-infrabar over reverted infrabar, would have been expected to give the same results as the old infrabar, even if there is an effective wild-type allelomorph—since such an allelomorph should not be present in either the old or the derived types. The result of a test against double-bar was: control (old infrabar) 35.7 facets, new infrabar 33.6 and 35.4 facets, respectively (individuals counted, 51, 30, and 30). The expectation is clearly correct; there are no significant differences.

A new reverted double-infrabar was tested, and at the same time the reverted infrabar of the earlier experiments was used. These and wild-type were tested against double-bar. Forty females of each type were counted, with these results: wild-type, 43.8; reverted infrabar, 44.4; reverted double-infrabar, 43.2. The reverted infrabar differs from wild-type in the opposite direction from the earlier test, and none of the differences here seem significant.

Two new bars, both from double-bar over round, were tested in three ways, as shown in table 3.

TABLE 3

Facet counts.

	OVER B^dB^d		HOMOZYGOUS ♀		MALE	
	n	*M*	*n*	*M*	*n*	*M*
Bar (control)	45	37.4	25	61.8	30	69.8
First derived bar	45	38.2	25	54.4	30	67.6
Second derived bar	45	35.4	25	49.6	60	61.0

Both of the derived types give lower facet numbers than the control, and in the case of the second derived type this difference is probably signi-

[2] The reverted double-infrabar and the first derived bar arose in the inbred forked stocks that were used in the earlier study. The other derived types were crossed at least five times to these stocks before counts were made. All the other stocks used in the present experiments were the same as those described in the earlier paper. The arguments for supposing modifiers to be alike are the same as in the earlier experiments. The temperature was 25° ± 1°.

ficant—but the difference is in the opposite direction from that found in the earlier experiments.

The conclusion seems to be justified that the observed differences are due to uneliminated modifiers or to chance environmental differences. In any case the new results do not at all support the hypothesis of a wild-type allelomorph affecting facet-number.

The three homozygous bar types recorded above averaged 55.3 facets; a double-bar over-round series of 40 females reared at the same time gave a mean of 43.8. This gives a position effect of the same kind as the data in the earlier paper, but rather less in extent.

REVERTED INFRABAR AND CROSSING OVER

Reverted bar or infrabar may be considered as a deficiency which is so short that it does not have the lethal effect of most deficiencies. It has been shown by BRIDGES (1917, 1919) and MOHR (1923) that females heterozygous for the usual type of deficiency give no crossing over in the section covered by the deficiency. This fact suggested another test of the relation between reverted bar and wild-type. It has been shown (STURTEVANT 1925) that any double-type over wild-type gives about 0.1 percent of single-type, the mutant individuals always being forked-fused crossovers. That is, in such heterozygous females there is crossing over between the two elements of the double-type. A test has now shown that reverted infrabar gives the same result as normal with double infrabar (table 4).

TABLE 4

Offspring from $\frac{B^{i}B^{i}}{f\,r_{ev}f_{u}}$ ♀ × $f\,r_{ev}f_{u}$ ♂.

0		1		2		TOTAL	MUT.
$B^{i}B^{i}$	ff_{u}	f_{u}	$f\,B^{i}B^{i}$	$B^{i}B^{i}f_{u}$	f		$f\,B^{i}$
1474	1415	1	3	35	42	2968	2

IS A WILD-TYPE ALLELOMORPH OF BAR NORMALLY PRESENT?

The results reported in this paper indicate that reverted bar and reverted infrabar do not differ from wild-type in their effect on facet-number or in their crossing over relations in heterozygotes with double-types. No other kind of test appears to be available, so the simplest conclusion is that ordinary stocks carry no allelomorph of bar. This is consistent with the fact that the change from round to bar has been detected only once in *D. melanogaster*, and no similar mutation has occurred in any other species.

One may surmise that the stock that gave rise to the original bar mutation had some sort of allelomorph already present—though other assumptions may also be made. It should be noted that, if a normal allelomorph were present in a stock and underwent unequal crossing over, as bar and infrabar do, the only stable condition would be that in which no bar allelomorph was present. While unequal crossing over does not alter the number of bar allelomorphs present in a stock, any strain without such allelomorphs will breed true, while any strain in which they are present will still give occasional individuals lacking them. If it be granted that Drosophila populations show great fluctuations in numbers, and often are reduced to very few individuals—as is very probable from their habits—then it would, in fact, be expected that unequal crossing over would have reduced most strains to the "no-bar" condition.

SUMMARY

1. "Mutation" at the bar locus interferes with crossing over in the neighboring garnet-forked interval, indicating that unequal crossing over at the bar locus occurs at the same time and by the same mechanism as normal crossing over.

2. Homozygous bar or infrabar stocks probably give equal numbers of reversions and of double-types; but the latter are somewhat less viable and much less easily detected, so that reversions are found more often. Improved technique has, however, decreased the observed difference.

3. The dominant mutant types Delta and Hairless show no "position effect" on bristle number—that is, $\Delta H/+$ and Δ/H do not differ in bristle number. This and other evidence suggests that relative position of genes is not usually significant in development—though new data agree with old in showing that it is significant in the case of bar.

4. New data indicate that reverted bar (or reverted infrabar) is not different in facet-number from wild-type, and that derived types in general show no effects of their past history.

5. Double-infrabar over reverted infrabar gives infrabar.

6. The last two points indicate that reverted bar (or reverted infrabar) is not different from wild-types, and the conclusion is drawn that ordinary wild-stocks carry no bar allelomorph.

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LINKAGE STUDIES WITH THE TOMATO.
II. THREE LINKAGE GROUPS

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INTRODUCTION

In the first study of this series (MACARTHUR 1926) nine of the most distinct qualitative characters of the tomato were tested in 24 different combinations, two at a time, for evidence of linkage. F_2 populations gave definite proof of the association of four of the controlling genes in one compact linkage group, and strong evidence of the independent assortment of the five remaining genes. There were thus established with reasonable certainty one linked group and basic "markers" for five others. This main conclusion still accords completely with all the well tested genetic data in the literature, and includes and confirms whatever linkages of qualitative characters have been determined by others (HEDRICK and BOOTH 1907; CRANE 1915; and LINDSTROM 1924–1927), but does not confirm the suspected linkage, between factors for yellow foliage and bilocularity, mentioned by PRICE and DRINKARD (1908) and JONES (1917).

The much-needed support of adequate backcross data, involving the same factors, has now been in large measure supplied. This new data, so far as it is merely confirmatory, is being published elsewhere, and will be cited in this paper only when concerned in some direct and essential way with the linkages to be described.

The internal relations existing between the members of the first linked group may now be stated more accurately, and the backcross recombination values given for all the possible pairings of factors. Two additional linkage groups, newly discovered with the help of mutant characters not hitherto employed in such work, are also described.

This account may be regarded in and for itself, but especially as foundation-building for succeeding studies on the most quantitative genetic factors, on mutation nature and frequency, and on the special genetics of types with changed chromosome numbers.

THE FIRST LINKAGE GROUP

Genes Dd, Pp, Oo (=P_r p_r), and Ss

The genes at the four loci known to be occupied in the first established group are mostly well known ones, already described (see especially PRICE and DRINKARD 1908; CRANE 1915; LINDSTROM 1924–1926; and MACARTHUR 1926). They are the differential factors controlling contrasting types of:

1. Stature. Tall (*D*) vine is dominant over dwarf (*d*). Nearly all, if not all, of the numerous dwarf varieties (included in the "Validum" subsection of the species by BAILEY 1896, p. 479), with their characteristic short, stiff, thick-set, tree habit and invariably associated dark green, rugose foliage, are probably derived ultimately from the same original "Laye Upright" mutant (VILMORIN-ANDRIEUX 1920), which appeared in a French garden about 1850. Their intercrosses have yielded only dwarf type plants, though these often differ interestingly in degree. But dwarf is to be distinguished from one "brachytic" sort, bearing normal foliage.

2. Fruit skin pubescence. Smooth (*P*) is dominant over peach (*p*).

3. Fruit elongation. Short (P_r or *O*) is partially dominant over elongate (p_r or *o*). To explain the multitude of varied and true-breeding shapes of tomato fruits certainly requires that one invoke the assistance of a considerable number of factors, factor combinations, and factor interactions. Prominent major factors, whose effects on shape have been isolated and identified more or less satisfactorily, are: a pair controlling the chief differences between non-fasciation and fasciation and thus affecting both locule number and shape; and two or three allelomorphs controlling degrees of elongation of the fruit. The exact mutual interrelations of these two sets of gene effects are not always entirely clear, but most of the difficulty presumably lies in our ignorance as to the number, manner of action, and precise effects of other modifying major and minor shape factors.

In any case there is no doubt that to the first linked group belong the

factors regulating relative length of fruits, that is the width/length ratio, as it varies from the somewhat flattened (oblate and peach), through the nearly spherical sorts (as in Apple, and Cherry), to those of more elongate contour (oval, Plum, Fig, Pear, etc.). Whether the factor causing constriction of neck in pear and calabash forms also belongs here, or is merelv a modifier with another locus, should be settled by crosses already made. For the present purpose a single major elongation factor ($p_r = o$) may safely be considered as the factor characteristic of all of the related fruit shapes grouped by BAILEY (1896) in the "Pyriforme" subsection of the species; as such it is partially recessive to one or more allelomorphs ($P_r = O$) in round and oblate sorts. These factors exhibit like linkage relations and values.

4. Inflorescence types. The usual simple cluster (*S*) is dominant over the compound, much-branched (*s*) type, carefully described and studied by CRANE (1915).

To the above should possibly be added rather uncertain and unlocated fruit size factors (LINDSTROM 1926b) and, even more doubtfully, a pair that may control resistance or susceptibility to western yellow blight (LESLEY 1926a).

Linkage relations within the first group

Tall-dwarf (*Dd*) and Smooth-peach (*Pp*) genes

The exceedingly close linkage (repulsion) shown by the total absence of the double recessive class in F_2 populations, numbering 677 plants, was noted again by LINDSTROM in a backcross generation, where it appears (1926b) that among 119 plants only four (3.4 percent) represented crossovers.

Tall-dwarf (*Dd*) and Round-elongate (pyriform) (P_r p_r or *Oo*) genes

Evidence for fairly strong linkage, with 11–20 percent crossing over was found in various F_2 populations in both coupling and repulsion phases. The shape phenotypes were again classified, as in the earlier work, by caliper measurements in both axes and by weights (yet unpublished). The evidence for linkage was sustained by the occurrence among 257 backcross progeny of 31 recombinations (12 percent). Their relative frequency in the reciprocal backcrosses demonstrates further that approximately equal crossing over takes place in microsporogenesis (13.2 percent) and in macrosporogenesis (11.7 percent) (table 1).

TABLE 1

Data from back crosses showing repulsion between d and o genes.

BACKCROSSES ♀ ♂	CLASSES				PERCENT CROSSOVERS
	Tall		Dwarf		
	Flat-round	Long-pear	Flat-round	Long-pear	
dd oo×F_1	5	28	18	2	13.2
F_1×*dd oo*	12	97	83	12	11.7
Total	17	125	101	14	12.06

By way of comparison, the similar more recent data of LINDSTROM (1927), obviously collected by use of the same criteria in classification, show a satisfactory agreement with 10.9 percent recombinations. Totals from both sources thrown together give 70 recombinations among the 616 plants of the backcross generation, that is, 11.4 percent crossing over in general, 11.9 percent in the male and 11.0 percent in the female.

Smooth-peach (*Pp*) and Round-long (pear) (*Oo*) genes

Ratios noted from 700 F_2 plants segregating for these genes suggest (Q = −.9) about 14–15 percent crossing over, allowing parity for the sexes. The F_2 findings are supported in essentials by backcrosses, which, however, point to an even stronger linkage (8.6 percent crossing over), since, among 280 plants, most bore smooth pear or peach round fruits, and in 24 cases only did new combinations appear (table 2).

TABLE 2

Backcross data showing linkage (repulsion) between p and o genes.

BACKCROSSES ♀ ♂	CLASSES				PERCENT CROSSING OVER
	Smooth		Peach		
	Round	Long	Round	Long	
pp oo×F_1	8	109	115	12	8.2
F_1×*pp oo*	4	14	18	0	11.11
Total	12	123	133	12	8.57

Round-elongate (*Oo*) fruit and Simple-compound (*Ss*) inflorescence genes

From the cross, Yellow Pear (*oo SS*)×Grape Cluster (*OO ss*) the F_1 hybrids bore practically round fruits in simple clusters, and the 259 F_2 progeny fell into classes showing about 20 percent crossing over in both sexes. Two hundred and ten backcross plants confirmed this value (table3).

TABLE 3
Data showing linkage between s and o genes.

GENERATION	CROSSES	CLASSES				Q VALUE	PERCENT CROSSING OVER
		Simple		Compound			
		Round	Long	Round	Long		
	(repulsion phase)						
F_2	Y. Pr.×Gr. Cl.	126	66	63	4	−.784	20
B. Cr.	*ss oo*×F_1♂	23	83	85	19		20
	(coupling phase)						
	CRANE's data (1915)						
F_2	W.I.×L.P.	91	16	11	13	.80	23

This determination of linkage value brings into line and explains reasonably what is apparently the last isolated case of linkage in the literature of tomato genetics—the hitherto unrelated results obtained by CRANE (1915) from his cross of Wonder of Italy with Lister's Prolific. The former variety bears oval fruits in compound clusters, the latter nearly spherical fruits in simple clusters; and CRANE states that the F_2 ratios "suggest partial coupling . . . . between the factor for simple inflorescence and that for short fruit" but that the numbers were too limited to estimate its intensity, or even to establish the linkage with certainty. But the association coefficient (Q = .8) and ratio fitting demonstrate that such a ratio is made logical if 20–23 percent crossing over occurs. Quite evidently the gene for long (pyriform) fruit and that for long (oval) fruit occupy the same locus, if indeed they be not identical.

Smooth-peach (*Pp*) and Simple-compound (*Ss*) genes

From the cross of Pink Peach (*ppSS*) with Grape Cluster (*PPss*) the smooth-skinned and simple-clustered F_1 plants have so far produced 195 F_2 individuals in the repulsion ratio: 104 *PS*:32 *Ps*:41 *pS*:7 *ps*, suggesting something over 30 percent crossing over. That this value is too high is indicated by the much more dependable backcross ratio; 184 plants from the double recessive F_2 ♀ ×F_1♂ were distributed in the proportion: 19 *PS*:71 *Ps*:68 *pS*:26 *ps*, the recombination value falling at 24.46 percent.

Tall-dwarf (Dd) and simple-compound (Ss) genes

The double recessive, dwarf compound (*ddss*), extracted from the earlier cross, when pollinated by the F_1 hybrid produced the repulsion backcross ratio: 21 *DS*:36 *Ds*:40 *dS*:9 *ds*, indicating 28.3 percent crossovers. But the considerable deficiency of the last class is to be noted.

Summary of crossover value

In table 4 are summarized from the sources mentioned above, the crossover values obtaining between the genes of the first linkage group.

TABLE 4

	d	*p*	*o*(p_r)	*s*
dwarf	..	3.4	11.4	28.3
peach		..	8.57	24.46
oval-pear			..	20.0
compound				..

The summaries (table 4 and figure 1) suggest that linkage group organization in the tomato resembles closely the classical Drosophila picture. Observed recombination percentages approximately correspond to map distances up to about 12 units; but fall short more and more for the greater distances, where double crossing over presumably occurs. Considering the best interpretation of the linkage values, it seems likely that the order of the genes in the chromosome is, therefore: *d*-*p*--*o*(p_r)----*s* and that the map loci stand at about 0.0, 3.4, 12.0, and 32+units respectively from the left end. But before stating these relations with the proper finality, further corroboration is desirable; hence the decision to await the maturing of results in several 3-point tests and the one 4-point test. Should these uphold the conclusions tentatively adopted, then the mapping of this chromosome will have been begun.

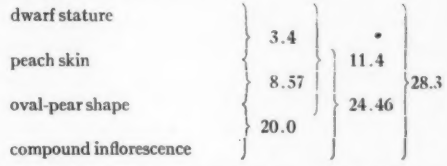

FIGURE 1.—Plan to show the probable linear order of genes of the first group and the observed percent of crossing over between them in their different pairings.

A SECOND LINKAGE GROUP

Description of genes Ll and Uu

The factors here involved are two simply mendelizing pairs, both concerned with chlorophyll coloration, one affecting the amount of the green pigment in general, the other the localized distribution of the color on the green fruits:

1. Color of foliage and immature fruits—*Ll* genes (heretofore designated as *Gg*). The normal full green color of foliage and young fruits (*L*) is dominant over the gradually yellowing, "lutescent" foliage (*l*) with its associated pale yellowish-white unripe fruits.

2. Distribution of green color over the immature fruits—*Uu* genes. The usual darker green stem-end coloration covering the basal third or fourth

of unripe fruits (*U*) is dominant over a mutant type found in one English variety, Devon Surprise, whose fruits have a perfectly uniform pale-green coloration from stem to stylar star (*u*). This lack of pattern, or self-colored pale condition, is a difference distinctly visible on green fruits of all sizes, is not masked or obscured appreciably by the presence of any other genes so far studied, even the pair *Ll*, and has no associated gene effects apparent in the color of ripened fruits or elsewhere in the plant.

Genetically this new condition is recessive to the normal in hybrids and is recovered pure-breeding in about 25 percent of the F_2 generation plants. The numerous crosses made with Devon Surprise have so far yielded an F_2 ratio of 914 plants bearing fruits with the dark butts to 297 with the uniformly colored fruits; and two sets of backcrosses have produced 166 dark: 152 uniform colored.

Genetic relations of Ll and Uu to other genes studied

That the genes, *Ll*, controlling foliage color, are independent in inheritance from the genes of the first linked group and from those controlling fruit flesh color (*Rr*), fruit skin color (*Yy*), fruit fasciation (*Ff*), and leaf shape (*Cc*) is indicated by data published (MACARTHUR 1926) and unpublished. Crosses and backcrosses of Devon Surprise with the linkage testers have demonstrated the same independent assortment between *Uu* and these genes; these negative data are being published in another place. In only one combination of genes, that is of *Uu* and *Ll*, was evidence of linkage obtained.

Linkage relations within the second group

In the exceptional case, where Devon Surprise (*uuLL*) was crossed with a linkage tester containing, among others the *UUll* genes, the normal type F_1 hybrids produced in the F_2 generation the significant ratio: 68 green foliaged with dark fruits: 33 lutescent with dark fruits: 22 green foliaged with uniform-colored pale fruits: 2 lutescent with uniform-colored pale fruits. The last two classes, those with self-colored pale fruits appear to have too low values, but whether correction is made for this or not the calculated coefficient of association gives the same figure, $Q = -.6845$, indicating a crossover percentage of 26+percent between *Uu* and *Ll* genes.

Since a supposed linkage between factors controlling foliage color (*Ll*) and those controlling locule number (*Ff* or others) is persistently, and apparently erroneously, cited in genetics texts, I offer here one sample of the abundant data, showing the complete independence of these character pairs. One cross involving these pairs has produced F_2 plants as follows:

218 green-foliaged with an average of 3.244 locules and 61 lutescent with an average of 3.25 locules. Nor is there any evidence of linkage between *Uu* and *Ff* genes, as would be expected if the admittedly very doubtful linkage based on the segregation of only 24 F_2 plants (PRICE and DRINKARD 1908) were real. But, until their experiment is repeated with the original varieties used I would not presume to imply that such an association does not exist, improbable though it now seems.

A THIRD LINKAGE GROUP

Description of genes Ff and Aa

The factors associated in this group are those controlling the widest differences in fruit fasciation (*Ff*), and a newly tested pair (*Aa*) controlling anthocyanin color development.

Fruit Fasciation

Non-fasciated fruits (*F*) of smooth outline and containing few cells, are dominant over fasciated fruits (*f*) of rough, irregular contour, containing usually 8–12 or more cells, some central in position; and when hybridized the F_2 populations segregate to give a simple 3:1 ratio. Never in our very numerous crosses have we found a 9:7 or other ratio indicating control of fasciation by two pairs of complementary factors such as WARREN (1925) reported.

Anthocyanin pigmentation

Usually the aerial portions of tomato plants, especially the stems and leaf veins, are distinctly purplish due to the presence of the sap pigment, anthocyanin. But an anthocyanin-less mutation (NORTON 1910) appeared in an inbred strain of Red Cherry tomatoes, as observed after its occurrence, by T. H. WHITE, when "a flat of seedlings produced a number of plants with entirely green stems." These green-stemmed plants bred true, and some of their purple sibs produced both purple and green progeny in a 3:1 ratio. Thirteen-year old seed of the mutant, kindly supplied to the writer, germinated well despite its age; thus reestablished, the green stem type behaved in crosses as a simple recessive to purple, and segregated pure in a ratio of 294 purple: 104 green plants. Winter-grown in the greenhouse, it is comparatively easy to distinguish heterozygote purples from pure purples, and these classify according to a 2:1 ratio.

Genetic relations of Ff and Aa to other genes studied

When tested for linkage, both the fasciated and the green-stemmed factors have given F_2 ratios fitting well to 9:3:3:1 in all combinations with

the genes of the first linked group and with those controlling foliage color (*Ll*), fruit flesh color (*Rr*), fruit skin color (*Yy*) and shape of leaf (*Cc*).

Linkage relations within the third group

Crossing the "Green Stem" mutant with its small, 2-celled, round Cherry type fruits, with pollen from a purple-stemmed linkage tester, bearing large, irregular fasciated fruits, brings together *aaFF* and *AAff* genotypes. The purple-stemmed F_1 hybrids bore bilocular, round fruits, whose selfed seed produced in the second hybrid generation: 80 purple stemmed and non-fasciated (mostly bilocular) (*AF*): 41 purple and fasciated (*Af*): 48 green and non-fasciated (*aF*): 1 green and fasciated (*af*). The repulsion between green stem (*a*) and fasciated (*f*) factors is as impressive in the field as it is obvious in the figures, and may be of some practical interest. By the coefficient of association ($Q = .913$) and ratio fitting the data is interpreted as showing about 14 percent crossing over, allowing an equal crossover frequency for both sexes.

In this as in the preceding instance of linkage I stress rather the establishment of the existence of the associations themselves, than any particular crossover values. Estimates from comparatively small F_2 repulsion groups are so liable to large error, due to the magnification of the chance fluctuations of the small double recessive class, that it is safer to resort to the easily-made backcrosses before assigning more precise values.

CONCLUSION

During the eight years that these linkage experiments have been in progress, over 35,000 plants have been grown and classified or studied in the various generations. Since chief attention was directed from the first on the presence of linkages and on linkage values quantitatively expressed, a considerable bulk of data has accumulated which, along with the pertinent facts from the literature, seem to lead to a few definite conclusions as to the organization of the germinal material in the tomato. An attempt has been made to state these conclusions conservatively and fairly in the resumé below, where are shown the genes determined in the linkage groups I, V and VI, and the "markers" assigned to groups II, III and IV, the group numbers being left the same as in the earlier work (MACARTHUR 1926, p. 401).

Until 3- and 4-point tests or suitable backcrosses are completed, no claim is made that the genes of group I are arranged in their final correct order, or properly spaced for mapping the chromosome, or that factors in

groups V and VI are more than roughly located; such an order and such unit distances afford the best interpretation of the data from all present sources.

I	II	III	IV	V	VI
0.0 dwarf	Yellow flesh	Clear skin	Potato leaf	0.0 fasciated	0.0 lutescent
3.4 peach				14.0 green stem	26.0 uniform (pale)
12.0 oval-pear					
32.0 compound					

SUMMARY

1. Adequate new linkage test have substantiated the earlier conclusion that in the tomato, linkage exists between factors differential for: *tall-dwarf* (*Dd*) stature, *smooth-peach* (*Pp*) skin, *spheric-elongate* (*pear* or *oval*) ($Oo = P_r p_r$) fruit shape, and *simple-compound* (*Ss*) inflorescence.

Within this *first linkage group* (group I) the linear order of genes, not yet determined by a 4-point test, is believed to be: *d-p--o*(p_r)------*s* (table 4, p. 415); if so the crossover values correspond closely to map distances up to nearly 12 units and fall short for greater distances, suggesting a chromosomal organization after the classical type (figure 1).

2. A new *linkage group* (group VI) contains two factor pairs, one (*Ll*, formerly called *Gg*) controlling green *versus* yellow ("lutescent") foliage color, and the other (*Uu*) regulating the distribution of chlorophyll color over immature fruits (see page 415).

Between *Ll* and *Uu* an F_2 repulsion ratio was obtained indicating probable linkage with about 26 percent crossing over. This still requires confirmation in backcrosses.

3. Another new *linkage group* (group V), also containing two factor pairs, is described. Genes (*Ff*) for non-fasciated *versus* fasciated fruit are closely linked (14 percent crossing over) with genes (*Aa*) controlling purple (with anthocyanin) *versus* green (without anthocyanin) stem.

4. The available linkage data is summarized on p. 419 showing the three established groups and the independent "markers" for three others.

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SEX-LINKED CHARACTERS OF POULTRY[1]

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INTRODUCTION

The sex-linked group of characters in poultry is of interest for several reasons. Some members of this group were not only among the first characters of the domestic fowl to be subjected to genetic study but were also among the first recognized examples of sex-linked inheritance. Barring and gold are two of the earliest recognized and best known characters of this group. Although a number of autosomal characters are well known, no linkage groups other than those whose genes are in the sex-chromosome are yet well established. The comparatively large number of sex-linked characters is also of interest since cytologists have shown that the sex-chromosome is by far the largest chromosome of the group characteristic of this species.

A more accurate knowledge of the linkage relations of characters of the sex-linked group is of interest in its relation to the genetics of this animal. The relations of this group of characters also have considerable practical interest since, through linkage it may be possible to handle, more effectively, the more or less intangible quantitative characters such as egg production and size.

METHODS AND MATERIAL

The study here presented was made during the period of 1923 to 1927 at the KANSAS STATE AGRICULTURAL COLLEGE. The material used was a part of the regular Experiment Station flock of this institution.

[1] Contribution number 43 from the Departement of Poultry Husbandry, KANSAS STATE AGRICULTURAL COLLEGE.

The breeds used in the study were the Single Comb White Leghorn, Jersey Black Giant, Silver Penciled Rock, Brown Leghorn, Buff Leghorn and Barred Plymouth Rock. By previous studies (HADLEY 1913) the Single Comb White Leghorn breed had been shown to carry the factor for barring and the writer (1924) has shown that this breed also carries the factor for rapid feathering. The Jersey Black Giant breed exhibits the extended black which is the allelomorph of barring and was found by the writer (1923) to possess the allelomorph of rapid feathering (slow feathering). This breed was also found to carry the determiner for dark shank. The Silver Penciled Rock, like most varieties of this breed, was found to carry the determiner for slow feathering and also to exhibit the silver color. The Brown Leghorn breed has been shown by GOODALE (1917) to carry gold and was found in this study to belong to the rapid feathering group. The Buff Leghorn was found to carry gold and rapid feathering while the Barred Plymouth Rock proved to be slow feathering and by previous studies (DUNN 1924) had been shown to carry the factor for silver. The symbol, B, has been used for barring, and b for its allelomorph; S for silver and s for its allelomorph; $S_l - s_l$ have been used for the pair determining rate of feathering, and $Y - y$ for the sex-linked shank color. For the breeds used, the genetic constitution with regard to the genes of the sex-chromosome may be symbolized as follows:

Single Comb White Leghorn	$BB\ s_l s_l\ YY\ SS$
Jersey Black Giant	$bb\ S_l S_l\ yy\ SS$
Silver Penciled Rock	$bb\ S_l S_l\ YY\ SS$
Brown Leghorn	$bb\ s_l s_l\ YY\ ss$
Buff Leghorn	$bb\ s_l s_l\ YY\ ss$
Barred Plymouth Rock	$BB\ S_l S_l\ YY\ SS$

In the earlier studies upon rate of feathering chicks were examined for the presence or absence of tail feathers at the end of the first, second, and third weeks. It was later found, however, that examinations made at ten days and three weeks were more reliable and in later studies records made at these ages were used for classification. All individuals were described at three different ages—one day, three months and six months. No attempt was made to classify chicks for shank color before the age of three months and descriptions made at this time checked very closely with the six-month description. Descriptions made at hatching were not found entirely reliable for classifying chicks for the gold and silver colors, nor for barring. So wherever possible the six-month description was depended upon for classification. Earlier descriptions were utilized in cases of death before maturity and were also used to check records made at the later

period. For all records chicks were identified only by wing-band number so that the experimenter's judgment would not be biased by a knowledge of the expected results.

FACTORS STUDIED

Four genes of the sex-chromosome were considered. Gene *S* and its allelomorph determine the presence of the gold or silver color of the plumage. Gene *B* together with its allelomorph determine the expression of barring of the feathers. Another gene of the sex-chromosome is S_l which, with its allelomorph, determines the age at which the chick down is replaced by feathers. The fourth gene studied is *Y* which affects the pigmentation of the shank.

The characters gold and silver have been found to be somewhat unsatisfactory ones with which to work since they are expressed only in regions where melanic pigment is absent. For plumage patterns where the black pigment has rather wide extension the classification of this character is sometimes difficult. In crosses of several breeds, the writer has observed the appearance of a yellowish to reddish brown color which usually appears upon the shoulders of males and breasts of females. In males this color is sometimes extended to the neck region. This color has been observed upon males which were known to be of the *Ss* composition and in some cases has led to confusion in classifying the phenotypes. The appearance of this color upon birds carrying *S* would sometimes lead to erroniously classifying them as homozygous golds.

The factor for barring has also been found difficult to study especially in cases where there are involved other pattern factors which restrict the extension of melanic pigment. This difficulty was encountered in the cross between the Barred Plymouth Rock and the Buff Leghorn since the latter carries the Columbian pattern which restricts the black pigment to the neck region and to the extremities of the tail and wings.

The gene for rate of feathering was found not to be interfered with by any other factors. All chicks not showing definite tail feathers upon the tenth-day examination were classed as slow-feathering. This character is fairly clean-cut in its expression and there is very little error in classification. A few exceptions were found in which birds known to be heterozygous for this factor, were rapid feathering. One such male when tested by breeding proved his heterozygous constitution. So although there is an occasional failure of the dominance of slow feathering this does not occur sufficiently frequently to cause any serious error in classification.

The gene which determines shank color is also very definite in its expression but is probably masked by certain plumage colors. The contrasted characters were yellow and blue shank. Although SEREBROVSKY considers the association of certain shank and plumage colors to be a matter of close association of their genes on the chromosome, the writer is of the opinion that the apparent linkage of yellow shank to barring and dark shank to black plumage is merely an instance of the extension to the shank of the effects of the factor determining the pigmentation of the plumage. The evidence supporting this belief will be discussed later in this paper. Because of their masking effects certain plumage color classes were eliminated in the shank color studies.

EXPERIMENTAL RESULTS

For study of the linkage relation of rate of feathering with shank color and barring, the cross of the Single Comb White Leghorn by the Jersey Black Giant was utilized. Although the relationships of three genes were followed in this cross, it could not be made a three point experiment because of the masking effects of some of the characters involved. White plumage color interfered with the expression of barring and its allelomorph black. Also, barring and its allelomorph are believed by the writer to mask the shank colors. Thus the white segregates were classified for linkage relations of rate of feathering to shank color, while the colored segregates were grouped for the study of the linkage of barring to rate of feathering. This selection of material for the two studies is allowable since it is based upon the segregation of an autosomal character which should in no way affect the ratios of sex-linked characters.

Linkage of rate of feathering and shank color

For determining the relation between these two factors four different F_1 males were used and the results were from three different breeding seasons. One male was used for two succeeding seasons. The four males were mated with 38 females. The females were pure bred Jersey Black Giants, pure White Leghorns, and segregates from the cross. It was only where the females carried both recessive factors that the male offspring could be included in the data. This accounts for the fact that the females in table 1 greatly outnumber the males. In this table are shown the data for linkage relations of rate of feathering to shank color, grouped according to the different males used.

Earlier work had shown slow feathering to be dominant to rapid and yellow shank to blue. Since the parental combinations were rapid feathering and yellow shank for the White Leghorn and slow feathering and blue

shank for the Jersey Black Giant the two crossover classes in table 1 are rapid-feathering blue shanks and slow-feathering yellow-shanks. It is seen that the numbers in the four groups are quite similar, there being

TABLE 1

Linkage relations of rate of feathering and shank color.

NUMBER OF F_2 MALE	FEMALES				MALES			
	Rapid yellow	Rapid blue	Slow yellow	Slow blue	Rapid yellow	Rapid blue	Slow yellow	Slow blue
887M (1924)	12	6	21	15				
(1925)	6	4	6	5		1	1	
935M	33	29	29	28				
911M	14	10	17	17				
957M	13	7	10	12	16	7	15	13
Total	78	56	83	77	16	8	16	13
Grand total both sexes	94	64	99	90				

Crossovers	163
Non-crossovers	184
Crossover percentage	47.0

some shortage in the one crossover group, rapid-blue. The calculated crossover percentage is 47. This closely approximates independent assortment and shows that these two genes are widely separated on the sex-chromosome.

Linkage of rate of feathering and barring

In this study were used the same four F_1 males which were mentioned in the previous experiment. The colored segregates were classified for rate of feathering and the barred and non-barred condition. The white segregates were not included in these data since the expression of the barring factor is masked by the white factor. In table 2 will be found four groups—rapid-barred, rapid-non-barred, slow-barred, and slow-non-barred. Since rapid-barred and slow-non-barred were the combinations found in the parental stocks, the other two classes constitute the crossover groups. Here again since only a few of the females mated to the males being tested were of the double recessive constitution, the number of male offspring included is limited. The percentage of crossing over for the total figures in this experiment is 48.6. This value approximates very closely that of

the previous experiment and is what would be expected from situations of independent assortment. Thus the genes for barring and shank are about equally distant from the gene for rate of feathering.

TABLE 2

Linkage relations of rate of feathering and barring.

NUMBER OF F_1 MALE	FEMALES				MALES			
	Rapid barred	Rapid non-barred	Slow barred	Slow non-barred	Rapid barred	Rapid non-barred	Slow barred	Slow non-barred
887M (1924)	9	16	8	16				
(1925)	1	4	2	4		2	1	3
935M	11	5	2	9				
911M	9	6	4	3				
957M	3	5	4	1	1	6	5	4
Total	33	36	20	33	1	8	6	7
Grand total both sexes	34	44	26	40				

Crossovers	70
Non-crossovers	74
Crossover percentage	48.6

Linkage of rate of feathering and gold

For this study it was necessary to make use of a cross differing from that used in the previous experiments. The F_1 males tested for percentage of crossing over were the offspring of a cross of a Brown Leghorn male by Silver Penciled Rock females. Two males were tested by mating to 17

TABLE 3

Linkage relations of rate of feathering and gold.

NUMBER OF F_1 MALES	FEMALES				MALES			
	Rapid gold	Rapid silver	Slow gold	Slow silver	Rapid gold	Rapid silver	Slow gold	Slow silver
987M	74	20	3	50	59	16	2	38
989M	82	8	4	67	68	24	5	56
Total	156	28	7	117	127	40	7	94
Grand total both sexes	283	68	14	211				

Crossovers	82
Non-crossovers	494
Crossover percentage	14.2

females all of which carried both recessive factors. Some of the females were sisters of the two males, other were F_1 females resulting from crosses of Brown Leghorn to Buff Leghorns and Rhode Islands Reds, and a third group were pure Brown Leghorns. Since they all carried gold and rapid feathering their other factors are of no interest here. In the Brown Leghorn breed rapid feathering was associated with gold and in the Silver Penciled Rock, silver was associated with slow feathering. Thus in table 3, of the four classes—rapid-gold, rapid-silver, slow-gold, slow-silver—the two middle ones include the crossover individuals. Table 3 shows that the original combinations tend to remain unbroken, there being 494 non-crossovers to 82 crossovers. The percentage of crossing over for the total of all crosses is 14.2.

As was earlier stated, the writer experienced some difficulty in distinguishing the gold and silver genotypes. This was particularly true of the male segregates since many had the duck-wing pattern in which the black areas limit the expression of gold and silver to the neck and shoulder regions. There also appeared to be involved an additional reddening or bronzing factor which when present in silver individuals made them difficult to separate from the lighter golds. In the female the gold and silver were less restricted by black and little difficulty was experienced. Since the ratio of gold and silver males is about equal and closely approximates that of the females, it is believed that the errors of classification were not large.

EFFECT OF AGE OF MALE UPON CROSSING OVER FREQUENCY

Since in the data for linkage relations of rate of feathering to shank color and barring, one male was used for two succeeding years, there is opportunity to consider the effect of age of the male upon the crossover percentages. Male 885M had 54 offspring during his first breeding year and 21 during the second, from which the crossover percentages between genes S_l and Y could be calculated. During the same years he had 49 and 11 offspring from which the linkage relations of S_l and B were calculated. For his first breeding year this male gave a 50 percent crossover value and for his second year a value of 47.6 percent between S_l and Y. For the linkage relations between S_l and B he gave a crossover value of 49 and 54.5 percent for the same two years. The numbers involved here are too small to base any reliable conclusions, but since the results of the two crosses are conflicting, it cannot be said that there is any evidence that the difference in age between the first and second breeding year of a male influences the crossover frequency.

DOUBLE CROSSING OVER

One cross was made which involved three sex-linked factors. The interference of these factors, one with another, was not so great but that some estimate of the degree of double crossing over could be made. This was a cross of the Buff Leghorn and Barred Plymouth Rock breeds. The former was of the composition $s_l\ s\ b$ and the latter $S_l\ S\ B$. The shortage of certain classes indicates that there were errors of classification in these data, but since the single crossover values, excepting those for S_l and S, are similar to those from other experiments, the results are here presented. The shortage of crossover between S_l and S will also reduce the double crossovers since these points are involved in double crossing over.

Non-crossover	65
Single crossover between S_l and B	39
Single crossover between S_l and S	4
Double crossover involving the above two	4

The above results are based upon the assumption that the genes are in the order S_l-S-B. The evidence, although not entirely conclusive, points to this arrangement. If the arrangement is S-S_l-B it would mean only a slight change in the last two figures. The foregoing results may be taken to indicate that double crossing over takes place relatively frequently in the sex-chromosome. With the same genes involved, SEREBROVSKY (1927) also found evidence of double crossing over in the sex-chromosome.

DISTRIBUTION OF FACTORS ON THE SEX-CHROMOSOME

With our present knowledge of the linkage relations of the factors upon the sex-chromosome of the chicken, it is of interest to map tentatively the genes of this chromosome. In addition to the relationships presented in this paper other workers have published data for the linkage relations between barring and gold. HALDANE (1921) from a limited amount of data (78 birds) first determined the percentage of crossing over to be 34.6. AGAR (1924) with a total of 56 birds, placed the crossover percentage at 46.4 for one cross and at 35.7 in another cross involving 28 individuals. SEREBROVSKY (1927) with a total of 77 individuals found the crossover value to be 43 percent. Most of the workers mention the difficulty of classification in studies of these factors. From the cross of Barred Plymouth Rock by Buff Leghorn the writer obtained a ratio 86 non-crossovers to 72 crossovers giving a crossover value of 45.6 percent. Due to the interference of the Columbian pattern with barring and barring with gold, it is felt that the ratio given is not entirely reliable.

However, the results of AGAR, SEREBROVSKY and the writer check very closely, 46.4, 43 and 45.6 percent. Each of the three above workers used a different breed as the carrier of gold.

In 1924 the writer gave the crossover value between rate of feathering and barring to be 47.1. Additional data slightly increased this value to 48.6 percent. SEREBROVSKY (1927) obtained a somewhat lower value, 44 percent.

The data available in 1924 led the writer to assign a crossover value of 48 percent from rate of feathering and shank color. Including additional data, the value given in this study is 47 percent. SEREBROVSKY (1927) gave for this same pair of factors a value of 49 percent.

SEREBROVSKY (1927) found the crossover value for gold and rate of feathering to be 19 percent. From a much larger number of birds the writer calculated the value to be 14.1 percent.

SEREBROVSKY (1927) has also attempted to determine the linkage relations of the shank color and barring pair of factors. He found two questionable crossovers among 69 individuals. He held that the genes from these two characters were quite closely associated. The writer's own data, showing these two factors to be about equally distant from the rate of feathering gene, would substantiate the suggestion that they are in the same general region of the sex-chromosome. It is doubted, however, that it is possible to determine the linkage relation of these two genes because of the interference of their characters. It is the opinion of the writer that what SEREBROVSKY considered as very close linkage is a situation of extension to the shanks of the effects of factors determining plumage pigmentation. The appearance of only dark-shanked black birds is due to the extension of the black pigment to the shanks as well as the feathers. The barring factor is held to be a partial inhibitor of black plumage pigment. This same factor might also inhibit black pigment of the shanks. It is true that most barred birds do show some black pigment on the shanks. However, all barred breeds of poultry have shanks which are predominately yellow. It might be held that the explanation for this lies in the existence of close linkage between barring and shank color. If barred breeds are yellow-shanked because of close linkage between shank color and barring, then barred breeds crossed to yellow-shanked breeds should produce only yellow shanks. Individuals of this breed are known to carry the factor for black plumage so if the yellow shanks are due to the inhibitive effect of the barring factor, then segregates released from the restrictions of this factor should have black shanks. A cross of

a Dark Brahma male by Barred Plymouth Rock females produced barred yellow-shanked sons and black plumage, black-shanked daughters. The Dark Brahma breed exhibits the silver duck-wing pattern in the male and the silver tri-penciled pattern in the female. The daughters of this cross would not carry the barring factor since they receive their only sex-chromosome from the father, who himself did not have this factor. Since the females here when released from the inhibiting effects of the barring factor are not only black color but black-shanked would seem to support the view that barred birds are yellow-shanked because of the inhibiting action of the barring factor, or more particularly that the extension factor influences shank color. The only difference between the sons and daughters of this cross is that the former carry the barring factor (and other sex-linked factors of the father) while the latter do not.

There are some black breeds (Black Leghorn) which have shanks that are predominately yellow. Individuals of the Black Leghorn breed, however, frequently show considerable black pigment on the shanks so the reduction of black here may be due to some other factor.

The sex-linked allelomorph of yellow shanks, which is blue, has been shown by BARROWS (1914) to be of this color because most of the pigment is dermal in location. The black pigment as seen through the epidermal layers appears dark blue. The shanks of black plumaged segregates are black and not blue. This is because birds of this color have the epidermal layer heavily pigmented. This being true, it can readily be seen that the pigmentation of the shank due to the extensive factor affecting black in the plumage would mask the sex-linked factor, the expression of which is largely in the dermal tissue. For the reasons given above, crosses involving both *Y* and *B* and their allelomorphs cannot be used for direct calculation of the map distance between these two genes.

SEREBROVSKY and WASSINA (1927) have suggested the probable arrangement upon the sex-chromosome of the four genes considered. They appreciated, as does the writer, that linkages as loose as those found cannot be used for accurate estimations of map distance. However, since we have information upon four different genes, and since the data presented by the various workers are in fair agreement, it is considered worth while to estimate roughly the positions which the accumulated data will indicate that these genes occupy.

Figure 1 shows the linkage relations determined by the various workers for the four factors considered. All studies have shown a high percentage of crossing over between rate of feathering and both barring and shank color. This would indicate that it is widely separated from these two fact-

ors and since its crossover value with each is about the same, these two factors are probably closely associated. Just how closely barring and shank color are associated, linkage as loose as those involved will

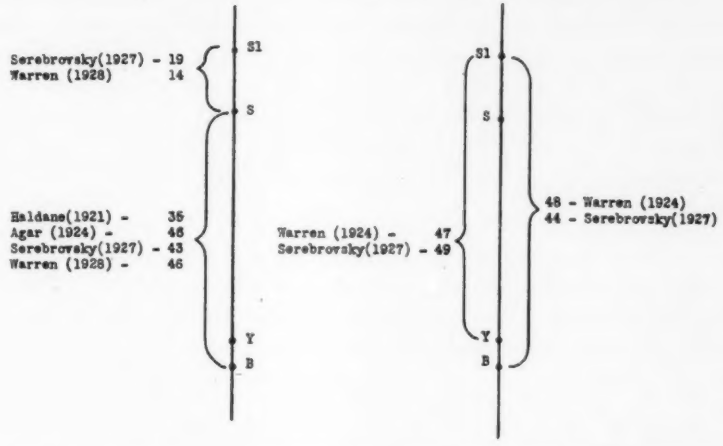

FIGURE 1.—Showing a tentative arrangement of the known genes of the sex-chromosome based upon crossing over percentages calculated by the various workers.

not indicate. Interference of characters will not permit a direct calculation of their crossover values. Thus the relative positions of barring and shank color with respect to rate of feathering cannot be reliably predicted and the tentative positions indicated for these two factors may quite possibly be reversed.

Since all data have shown that gold exhibits very loose linkage relations with barring, these two genes must be distantly separated. Since the crossover value ($14\pm$) between gold and rate of feathering is much lower than the value between it and barring ($45\pm$), gold and rate of feathering must be in the same end of the chromosome. Although the data are not entirely conclusive, gold has been tentatively placed between rate of feathering and barring. The data of the writer, which includes a larger number of individuals than those of all other workers combined, confirm the arrangement of genes suggested by SEREBROVSKY (1927). It can at least be said that genes *B* and *Y* are associated in one section of the chromosome and that genes S_l and *S* are about 14 units apart in a distant section of the same chromosome.

The linkage relations of gold to shank color have not yet been determined by anyone and the writer is not familiar with any pure breeds whose constitution is such as to make them suitable for determing these relations.

No other sex-linked characters have definitely been determined in poultry. PUNNETT (1923) mentions the black skin pigment in the Silky breed as exhibiting sex-linked behavior but suggests that it may be due to the action of the gene *Y*. LEFEVRE and RUCKER (1923) have listed the spangling pattern as belonging to this group, but other workers have not been able to substantiate their conclusion. So until other workable sex-linked factors of the fowl have been found the chromosome cannot be definitely mapped.

Probable errors have not been calculated for the crossover values given in the foregoing tables since it is believed that the error of map distance due to double crossing over is probably greater than that of random sampling. The error due to double drossing over cannot be estimated until intervening genes have been found.

SUMMARY

1. The crossover value between rate of feathering and shank color was found to be 47 percent.
2. Rate of feathering and barring gave a crossover value of 48 percent.
3. The factors for rate of feathering and gold gave a crossover value of 14 percent.
4. Gold and barring gave a crossover value of 45 percent.
5. The crossover value for the factors barring and shank color cannot be directly calculated because of masking effects.
6. Accurate mapping of the genes on the chromosome cannot be accomplished until more closely associated points are found.

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TRANSPARENCY AND MOTTLING, A CASE OF MENDELIAN INHERITANCE IN THE GOLDFISH *CARASSIUS AURATUS**

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INTRODUCTION

In the spring of 1924 I made a preliminary study of the heredity of goldfish by crossing various breeds of goldfish and mating domesticated breeds of goldfish with the wild goldfish. During the progress of this preliminary study I noticed that the inheritance of one of the characters under investigation was rather simple.

This character was called "transparent scale" in my previous paper (CHEN 1925). The goldfishes of this breed have only a few normal scales, the remainder of the body being apparently naked. The apparently naked part of the body is really covered by scales which, on account of the lack of a layer of reflecting tissue on their inner side, are as transparent as glass. This breed of goldfish is called "shubunkin" in Japan (MATRUBARA 1908) and is known as "calico" in the United States (INNES 1917).

Beginning in the spring of 1925 I started more extensive experiments to investigate the mode of inheritance of this character, "transparent scale"or "calico." In the summer of 1925 I obtained enough evidence to prove that the inheritance of this character is Mendelian. A preliminary report of this discovery had been made (CHEN 1926). The present paper is the final report of this investigation.

* The GALTON AND MENDEL MEMORIAL FUND contributes the accompanying colored illustrations.

MATERIAL AND METHODS

The materials of this investigation were the transparent scaled fancy goldfishes, the normal scaled fancy goldfishes, and the normal scaled wild goldfishes. The fancy goldfishes were obtained from a local goldfish breeder, the wild ones being obtained from the food-fish market of Nanking.

The fishes were bred and grown in water reservoirs made of burned clay. These reservoirs were made in the shape of a huge cup with the upper diameter about 38 inches, lower diameter about 19 inches, and a depth of about 32 inches. Such a reservoir is large enough to contain about eighty young fishes to a size capable of reproduction in the next breeding season.

Goldfish take one year for a generation. The breeding season of the goldfish at Nanking begins in the middle of April and ends in the middle of June, lasting about two months. During this period a single male fish may mate four or five times and a female fish two or three times with an interval of about ten days between successive matings.

I tried to make the different crosses of goldfish by means of artificial fertilization. These attempts failed. Hence all the various crosses in my experiments were made by the ordinary method of natural fertilization as employed by the common goldfish breeders except with some necessary precautions.

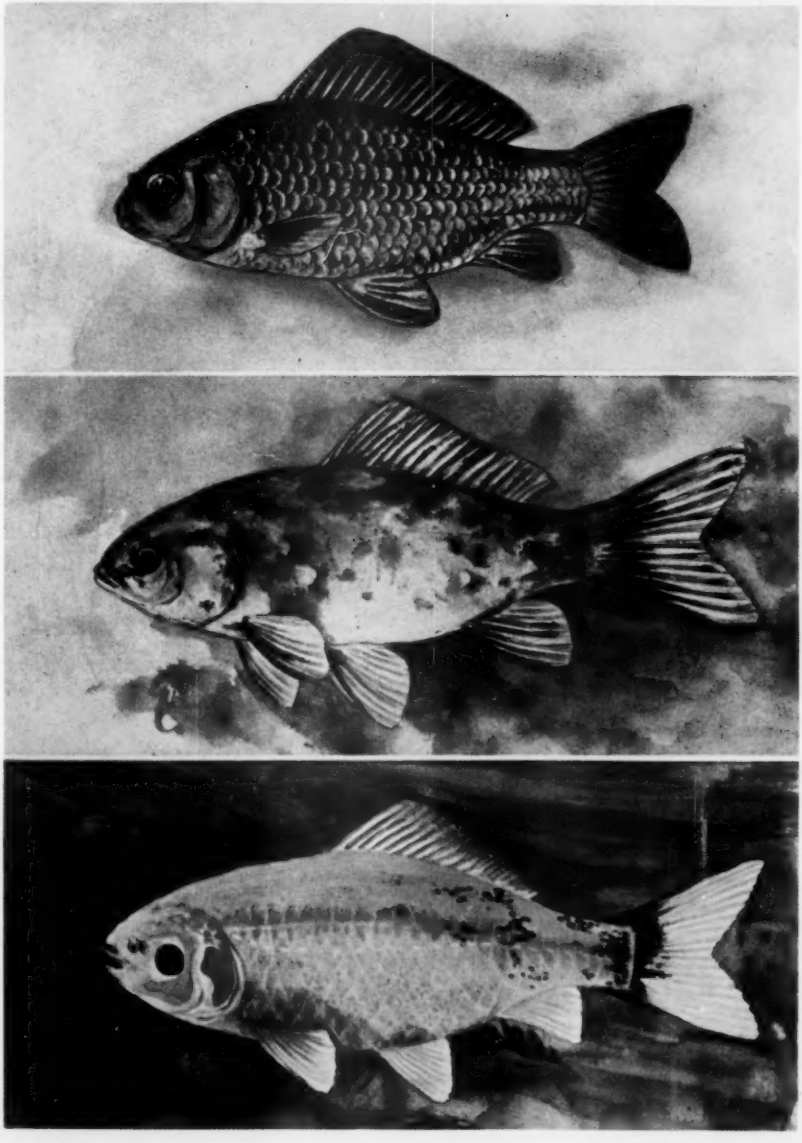
At the beginning of the breeding season I separated the female and male fishes into different reservoirs. The male fish is distinguished from the female by the presence of numerous small white tubercles, called the "pearl organs," along the front rays of the pectoral fins and on the external side of the two opercula. This separation of the females from the males was needed in order to avoid the loss of the eggs through the free matings in a reservoir as soon as the breeding season began and thus to insure the females to have eggs to lay when these were required for the experiments.

In mating goldfish the practice of the common breeders is to mate two or three female goldfishes with three or four males. Since such a practice would introduce some errors into the experimemts, I always mated one female with one male in a reservoir.

During mating the eggs of the goldfish were deposited on a bunch on water plant (Myriophyllum) which had been introduced into every mating reservoir. The eggs hatch from three days to a week after fertilization depending upon the temperature of the water in the reservoir.

LEGEND FOR PLATE 1

FIGURE 1.—Normal scaled fish.
FIGURE 2.—Heterozygous mottled fish.
FIGURE 3.—Homozygous transparent fish.



About two days after hatching, when the young fish were just able to swim freely, they were fed with finely pulverized yolk of boiled chick eggs. About three days later I began to feed the young fish with living cyclops and about two weeks after hatching the young fishes were fed with Daphnia. The goldfishes grew very rapidly on this diet.

About forty days after hatching the young fish had grown to about 25 mm in body length, being a size large enough to be examined with the naked eye. At this time the character of transparent scale had developed to such an extent that the fish with this character might be distinguished from the normal scaled fishes. The data concerning the offspring of the matings were mostly taken at this time. Sometimes the progeny of a single mating amount to about seven or eight hundred.

Frequently the young offspring were discarded after being examined, classified, and recorded. Sometimes these fishes were reserved for further examination. The young fishes at this time, about forty days after hatching, varied greatly in size. Goldfishes are cannibals and if the small fishes remain with the larger ones, the latter will devour the former. In a heterogeneous group of genotypically different classes of fish the quick growing will soon devour the slow growing and the former will survive while the latter perish. In order to avoid the introduction of this source of error into my experiments, I always separated the offspring of a single mating, at the age of about forty days after hatching, into two groups according to the size of the young fishes and reared these two groups separately in different reservoirs. Thus the slow-growing class had as good a chance to survive as the quick-growing class.

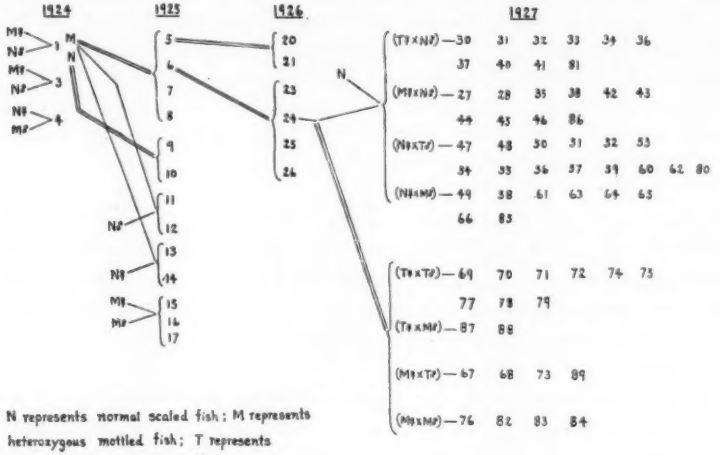
NARRATIVE OF THE EXPERIMENTS

1924. The parents and the F_1—In the spring of 1924 I made the following crosses (figure 1):

1. Transparent scaled fancy goldfish ♀ Normal scaled wild goldfish ♂
2. Normal scaled wild goldfish ♀ Transparent scaled fancy goldfish ♂
3. Transparent scaled fancy goldfish ♀ Normal scaled fancy goldfish ♂
4. Normal scaled fancy goldfish ♀ Transparent scaled fancy goldfish ♂

With the exception of the mating number 2, all of the matings produced offspring. The F_1 fishes of all the fertile matings were unexpectedly of two classes. One class of the offspring had transparent scales and mottled condition while the other class had normal scales. The normal scaled offspring of family 1 were exactly like the wild goldfish (plate 1). The transparent-scaled offspring of the same family were similar to the wild goldfish in every respect except with transparent scales and the mottled

pattern (plate 1, figure 2). The latter class of fish with a long body and unpaired fins resembles closely a Japanese breed of goldfish called "shubunkin" but is an entirely new form to the goldfish fanciers in China. I found both females and males among the transparently scaled and the normally scaled classes.



The two classes of offspring were of approximately equal numbers as shown by the data in table 5. This result was interpreted by assuming that the transparent scaled parents of the families 1, 3, and 4 were all heterozygous with respect to a pair of allelomorphic factors one of which when homozygous was responsible for the production of the transparent scales while the other factor, when homozygous, would cause the production of the normal scales. All the normal scaled parents were homozygous for normal scales. The gene for the production of the transparent scales is accordingly dominant to the gene for the production of the normal scales. Hence the transparent scales and the mottled pattern appeared on the body of the heterozygous fish and the offspring of the three families were of the two classes, heterozygous mottled fish and homozygous normal scaled fish.

1925. The F_2.— From the offspring of family 1 I made four matings between the transparent scaled females and the similarly scaled males. The offspring of these matings were, as expected, of two classes, transparent

scaled and normal scaled. The number of individuals in these two classes and their ratios are presented in table 3, families 5, 6, 7, and 8.

The data in table 3 follow closely the typical Mendelian ratio of the F_2 generation, three to one. The assumption that the original parents of 1924 were heterozygous and all the transparent scaled offspring were also heterozygous was thus confirmed.

In addition to the four matings mentioned above, I had also made four backcrosses between the transparent scaled offspring of family 1 and the normal scaled fancy goldfishes. The results of these matings are presented in table 5, families 11, 12, 13 and 14. The data of these back crosses also prove that the transparent scaled offspring were heterozygous as expected.

The matings between the normal scaled females and males of the offspring of family 1 had also been made. One of these matings produced 761 normal scaled offspring (family 9). Another mating produced 248 normal scaled offspring (family 10). Neither of these matings produced a single transparent scaled fish. This result is in accord with the assumption that the original normal scaled parents and the normal scaled offspring were homozygous and that the normal scaled condition is recessive.

1926 The F_2 generation—On account of a necessary absence from Nanking during the breeding season of 1926 I did not have time to make more extensive experiments than nine matings between the transparently scaled females and males of the F_2 generation.

Only six out of the nine matings produced offspring forming the families 20, 21, 23, 24, 25, and 26. Families 20, 21, 23, 25, and 26 consisted of transparent scaled and normal scaled offspring in the typical Mendelian ratio of three to one (table 3). Family 24, however, consisted of 346 transparent scaled fishes together with only one normal scaled fish. The exceptional normal scaled fish was thought to have been accidently introduced into this family from some other source due to the carelessness of the aquarium keeper during my absence from Nanking.

1927 The F_4 generation.—In the spring of 1927 I took twenty females and twenty males from the offspring of family 24 and backcrossed them with normal scaled goldfishes. I obtained the following results:

Nine transparent scaled females and thirteen transparent scaled males in mating with the normal scaled goldfish produced only transparent scaled offsprings. These twenty-two transparent scaled fishes were thus proved to be homozygous with respect to the gene for the transparent scale.

Nine transparent scaled females and seven transparent scaled males in mating with the normal scaled goldfishes produced offspring of two kinds in approximately equal numbers. These sixteen transparent scaled fishes were thus proved to be heterozygous, with one gene for transparent scale and one gene for normal scale.

Two transparent scaled females in the backcross with normal scaled goldfishes failed to produce offspring.

By the above mentioned breeding work I proved twenty-two transparent scaled fishes to be homozygous and sixteen transparent scaled fishes to be heterozygous. These two classes of fishes were examined in detail. I found that they have distinguishing features which although difficult to define in terms of words, are, on the whole, easily recognized as soon as it has once been noted. Figure 2 of plate 1 shows a heterozygous transparent scaled fish. Figure 3 of the same plate shows a homozygous transparent scaled fish. Detailed descriptions will be made later.

After I ascertained the genotypes of the thirty-eight transparent scaled fishes I made the following matings between the females and the males of these thirty-eight fishes near the end of the breeding season:

1. Nine matings between the females and the males of the homozygous transparent scaled fishes.
2. Two matings between the homozygous transparent scaled females and the heterozygous males.
3. Four matings between the heterozygous females and the homozygous transparent scaled males.
4. Four matings between the females and the males of the heterozygous fishes.

The above matings were made for the double purpose of testing whether the earlier assumptions were true and to see whether the ratios of the number of homozygous transparent scaled fishes to that of the heterozygous fishes were approximate to the expected ratios.

The results of these matings are presented in tables 1, 4, and 6. The data in these tables show that observed ratios are in close accordance with expectation.

TERMINOLOGY AND SYMBOLS

At the beginning of the present investigation I could distinguish only one kind of transparent scaled goldfish. This breed of goldfish is called "shubunkin" in Japan and "calico" in the United States.

After four years of experiments I found that the transparent scaled fishes could be separated into two classes, one of which is nearly completely

transparent while the other class always has a few normal scales and the characteristic mottled pattern. The former class is homozygous and the latter class heterozygous.

Since the homozygous transparent scaled fishes are phenotypically distinguishable from the heterozygous transparent scaled fishes, hereafter I shall call the fishes of the former class "transparent fish" and the fishes of the latter class "mottled fish."

The distinction between heterozygous mottled and normal fish is very sharp but the distinction between heterozygous and homozygous transparent fish is not so sharp and they even overlap when the fishes are young. According to the terminology of T. H. MORGAN (1919) a character is said to be recessive if the individuals with this character are sharply separable from the heterozygous individuals; a character is said to be dominant if the individuals with this character are overlapping with the heterozygous individuals. In this sense we may say that the "transparency" is a dominant character while the normal condition is recessive.

According to the methodology of T. H. MORGAN the symbol of the gene causing a dominant mutant character, like the bar eye of Drosophila, is a capital letter primed while the symbol to represent the gene causing the normal recessive character is the capital letter alone. Adopting this method of MORGAN I shall use the letter T to represent the gene causing the development of normal scales and the symbol T' to represent the gene causing the development of transparent scales.

DATA FROM BREEDING EXPERIMENTS

Normal scaled fishes breed true.—During the period of 1924 to 1927 I made numerous matings between the females and males of the normal scaled fancy goldfishes as well as the wild goldfishes. With the exception of a very few instances to be cited in a later section of this paper the offspring of all these matings consist of only normal scaled fishes. Furthermore I made two matings (families 9 and 10) of normal scaled fishes produced from a cross (family 1) between a transparent scaled fish and a normal scaled fish. One of the matings (family 9) produced 761 normal scaled fishes. The other mating (family 10) produced 248 normal scaled fishes. Both of these two matings did not produce a single fish with transparent scales.

Transparent fishes breed true.—In the spring of 1927 I made nine matings between the female and male transparent fishes. These nine matings produced many thousands of offspring, all of them were transparent fishes. The data are in table 1.

TABLE 1

Families which illustrate the true-breeding of transparency.

PEDIGREE NUMBERS			NUMBER OF OFFSPRING, ALL TRANSPARENT
Parents		Family	
♀	♂		
24(B5)*	24(C5)	69	81
(B6)	(C6)	70	183
(B7)	(C7)	71	41
(B8)	(C8)	72	122
(B10)	(C10)	74	150+
(B11)	(C11)	75	150+
(B4)	(C1)	77	100+
(B5)	(C2)	78	100+
(B6)	(C4)	79	100+
Total			1027+

* The number outside of bracket represents family number; those within the brackets represent individual numbers.

Transparent fish crossed with normal fish produce mottled fish.—In the spring of 1927 I made ten crosses between transparent females and normal males and fourteen crosses between normal females and transparent males. All these matings produced only fishes with the characteristic mottled pattern. The data of these matings are in table 2.

TABLE 2

Families which show the mottled heterozygous type in the F_1 of crosses between transparent and normal.

PEDIGREE NUMBERS		NUMBER OF OFFSPRING ALL MOTTLED	PEDIGREE NUMBERS		NUMBER OF OFFSPRING, ALL MOTTLED
♀ Parent*	Family		♂ Parent**	Family	
24(B4)	30	150+	24(C1)	47	350+
(B5)	31	174	(C2)	48	100+
(B6)	32	150+	(C4)	50	300+
(B7)	33	14	(C5)	51	300+
(B8)	34	100+	(C6)	52	200+
(B10)	36	80+	(C7)	53	100+
(B11)	37	150+	(C8)	54	100+
(B14)	40	100+	(C9)	55	300+
(B15)	41	14	(C10)	56	100+
(B10)	81	100+	(C11)	57	200+
			(C13)	59	200+
Total		1032+	(C14)	60	200+
			(C16)	62	200+
			(C5)	80	100+
* ♂ parents were normal scaled.			Total		2750+
** ♀ parents were normal scaled.					

Mottled fish never breed true, but always produce some normal fish and some transparent fish.—In the spring of 1925 I made seven matings between female and male mottled fishes. In the spring of 1926 I made five similar matings. The data of these matings are in table 3. These data show

TABLE 3

Segregations following mating mottled females with mottled males.

FAMILY NUMBER	TRANSPARENT AND MOTTLED OFFSPRING		NORMAL SCALED OFFSPRING		TOTAL NUMBER OF OFFSPRING
	Number	Ratio	Number	Ratio	
5	532	2.86	212	1.14	744
6	856	3.01	280	.99	1136
7	508	2.87	201	1.14	709
8	744	2.87	291	1.12	1035
15	482	3.08	143	.92	625
17	119	3.17	31	.83	150
20	236	2.84	96	1.16	332
21	181	3.07	55	.93	236
23	152	2.81	64	1.19	216
25	375	2.98	128	1.02	503
26	301	3.17	79	.83	380
Totals	4486	2.958	1580	1.042	6066
P.E.		±0.015		±0.015	

that among the offspring there were always produced some normal fishes. The ratios of the transparent and mottled fishes to the normal scaled fishes were all approximate to 3 to 1. The deviation of the observed ratios

TABLE 4

Segregations following mating mottled females with mottled males.

PEDIGREE NUMBERS			NORMAL SCALED OFFSPRINGS		MOTTLED OFFSPRING		TRANSPARENT OFFSPRING		TOTAL NUMBER OF OFFSPRING
Parents ♀	♂	Family	Number	Ratio	Number	Ratio	Number	Ratio	
24(B12)	24(C12)	76	71	.83	170	1.98	102	1.19	343
(B2)	(C3)	82	110	.82	274	2.18	120	.95	504
(B9)	(C12)	83	82	1.27	117	1.80	60	.93	259
(B12)	(C15)	84	82	1.00	168	2.05	78	.95	328
Totals			345	0.96	729	2.03	360	1.00	1434
P.E.				±0.03		±0.036		±0.03	

from this expectation is 0.042 while the probable error of the expectation is 0.015. Since the deviation is less than three times the probable error the fitness of the observed ratio to the expectation is reasonably close.

In the spring of 1927 I made four additional matings between female and male mottled fishes. The results of these matings are in table 4 which show that among the offspring there were always about one fourth of transparent fish, one half of mottled fish, and one fourth of normal fish. The observed ratio is very close to the expectation. In the case of the total normal to the total offspring the observation is in perfect agreement with the expectation. The deviations of the other two ratios are only about once times the probable error.

Mottled fish crossed with normal fish.—I made fourteen matings of mottled females crossed with normal males and eleven matings of normal females crossed with mottled males during the breeding seasons of 1924 to 1927. Among these 25 matings I obtained quantitative data in eleven of them. The data are presented in table 5. I had also made a rough exami-

TABLE 5

Mottled heterozygotes backcrossed to the normal.

FAMILY NUMBERS	TYPE OF CROSS	MOTTLED OFFSPRING		NORMAL SCALED OFFSPRING		TOTAL NUMBER OF OFFSPRING
		Number	Ratio	Number	Ratio	
1	M♀×N♂*	156	1.08	133	.92	289
3	M♀×N♂	33	1.12	26	.88	59
11	M♀×N♂	654	1.05	592	.95	1246
12	M♀×N♂	557	1.04	511	.96	1068
43	M♀×N♂	127	1.06	112	.94	239
86	M♀×N♂	23	.79	35	1.21	58
4	N♀×M♂	120	1.05	111	.95	231
13	N♀×M♂	146	.92	171	1.08	317
14	N♀×M♂	573	.99	580	1.01	1153
64	N♀×M♂	101	1.06	90	.94	191
85	N♀×M♂	62	1.02	59	.98	121
Totals		2552	1.03	2420	.97	4972
P.E.			±0.01		±0.01	

* N represents normal scaled fish; M represents heterozygous mottled fish.

nation of the results of the 14 remaining matings and found them to consist of about one half of mottled fishes and one half of normal scaled fishes.

The data in table 5 show that all the matings produced about one half of mottled fish and one half of normal fish. The observed ratio fits very

closely to the expectation since the probable error of the expectation is ±0.01 and the deviation of the observed ratio from the expectation is 0.03, being only three times the probable error.

Mottled fish crossed with transparent fish.—In the spring of 1927 I made two matings between transparent female and mottled male fishes and four matings between mottled female and transparent male fishes. The results of these matings are in table 6 which show that all these matings produced

TABLE 6

Mottled heterozygotes backcrossed to the transparent.

PEDIGREE NUMBERS			TYPE OF CROSS	MOTTLED OFFSPRING		TRANSPARENT OFFSPRING		TOTAL NUMBER OF OFFSPRING
Parents ♀	Parents ♂	Family		Number	Ratio	Number	Ratio	
24(B7)	24(C18)	87	T♀×M♂*	123	1.00	124	1.00	247
(B11)	(C19)	88	T♀×M♂	145	1.07	127	.93	272
(B1)	(C1)	67	M♀×T♂	24	.89	30	1.11	54
(B2)	(C2)	68	M♀×T♂	28	1.06	25	.94	53
(B19)	(C9)	89	M♀×T♂	138	.85	188	1.15	326
Totals				458	.96	494	1.04	952
P.E.					±0.02		±0.02	

* T represents homozygous transparent fish.

about one half of mottled fish and one half of transparent fish. The observed ratio fits very closely to the expectation since the deviation is only two times the probable error in the total offspring.

Besides the above observations families 24 and 73 had been roughly examined and found to consist of about one half of transparent fishes and one half of mottled fishes.

The character is not sex-linked.—During the progress of the breeding work I always paid attention to the problem of whether the character is sex-linked or not. Since the secondary sexual character, that is, pearl penis organ, of the male fish, is not visible before the fish is grown to considerable size I often dissected many young fishes to ascertain their sex. In all the various matings which I made I found that there were always female and male offspring in approximately equal numbers. Based upon all the facts which I observed in the breeding experiments I conclude that this character is not sex-linked no matter whether the inheritance of sex in fish be XY type, WZ type, or the modified XY type with genes on the Y chromosome as well as on the X chromosome as found in the case of *Aplocheilus latipes* by AIDA (1921).

THE MANIFOLD EFFECTS

I have already mentioned that by means of the progeny tests I had definitely found 22 transparent scaled fishes to be homozygous and 16 transparent scaled fishes to be heterozygous. With the two classes of transparent scaled fishes and the normal scaled fishes at my disposal I made a detailed examination of the various effects of the different combinations of the allelomorphic genes T and T'. This study is extended and confirmed by the examination of the offspring produced by the matings of the parents whose genotypic constitutions are definitely known.

Homozygous Normal.—With the presence of the gene T in duplex condition the fish has one or several layers of reflecting tissue covering the entire body including the head, trunk, and iris and excluding the pupils and the fins, being more abundant on the ventral surface of the body. The fish has also two kinds of chromatophores, that is, the black melanophores and the yellow xanthophores, distributed on the surface of the body and the fins and usually more abundant on the dorsal surface than on the lateral surface while on the ventral surface the chromatophores are generally absent.

With the exception of the breeds "brown" and "blue" the fishes with the gene T in duplex condition always have similar body color until about ten weeks after hatching, beginning in July, when the various brilliant colors of goldfish begin to appear.

Homozygous Transparent.—When the gene T' is in the duplex condition the fish has an appearance (plate 1, figure 3) entirely different from that of normal scaled fishes. The gene reduces the reflecting tissue so that it is either entirely, or nearly entirely absent on the body of the fish thus showing the red blood in the gills, the black pigment in the eyeballs, the white testis, the yellow eggs, and the black intestine visible to the eye without dissection. The melanophores are also greatly reduced to a black dotted area near the junction between the caudal peduncle and the caudal fin while on the other parts of the body the melanophores are either absent or, when present, form isolated dots very few in number. The presence of T' in duplex condition causes usually the complete loss of xanthophores. Occasionally xanthophores are present and form a yellow spot on one side of the trunk.

The expression of the genes varies in different individuals. Among the twenty-two homozygous transparent fishes the effects on the reflecting tissue varied as follows:

Five fishes were completely transparent, that is, without reflecting tissue altogether.

Fourteen fishes had a very small area of reflecting tissue situated on the ventral or the ventro-lateral parts of the abdomen.

Seven fishes had some reflecting tissue on one or both of the irises.

Four fishes had some reflecting tissue on a part of an operculum or a whole operculum but never on both opercula.

Three fishes had one to three normal scales. Nineteen fishes had no normal scale.

The effects on the melanophores and the xanthophores also varies in different individuals. Generally the melanophores are restricted to the junction between the caudal peduncle and caudal fin while the xanthophores are entirely absent. But I had also frequently observed that the melanophores are present on the basal area of the dorsal fin, on the basal area of the anal fin, or on the top of the head, and the xanthophores are present on a small area of the surface of the body usually beneath the dorsal fin.

Heterozygous Mottled.—The expression of the effects of a gene T and a gene T' in combination is more complex than when the genotypic constitution is TT or $T'T'$. The head is sometimes completely covered with the reflecting tissue, sometimes transparent in a small portion of the skin, or sometimes lacking the reflecting tissue in an entire operculum or even in an area as large as about one half of the surface of the head.

In the heterozygous mottled fishes there are always some normal scales mixed with the transparent scales. The number of normal scales in the 16 heterozygous fishes which I studied in detail varied from 2 to 18, being most frequently from 2 to 10.

In the body wall of the trunk the inner layer of the reflecting tissue is either entirely present or reduced to about two-thirds of the whole surface. Hence the heterozygous fish is never completely transparent.

The melanophores, xanthophores, and the bluish prismatic color are always mixed in the characteristic mottle condition and there is a greater number of the colored spots on the dorsal and the dorsolateral parts of the body than on the ventral parts.

WHY TRANSPARENT FISHES ARE RARE

In reviewing the books on goldfish published in Japan, such as that of MATRUBARA (1908), or in the United States such as that of INNES (1917), I found no records of the existence of the transparent fish as distinguished from the mottled fish, "shubunkin," or "calico." In the Chinese books on

goldfish I had also found no records of the transparent fish as a distinct breed. In my previous paper (CHEN 1925) I described such a fish but at that time I was not aware that such a fish might be different from the mottled fish in genotype and form a distinct breed. Altogether I mated nine transparent scaled fishes obtained from the local goldfish breeders at Nanking and found that all of them were heterozygous (families 1, 3, 4, 15, 16, 17). All these facts show that the transparent fish is rarely found in the goldfish aquarium of the ordinary goldfish breeders.

But in my breeding work I found that the proportion of the transparent fish in the F_4 generation is not less than one fourth. The transparent fish did not seem to have a poor viability as ordinary recessive breeds of animals or plants. I suggest the following three explanations to be the cause of the rarity of the transparent fish in the aquarium of the common goldfish breeders:

One explanation is that the heterozygous class being twice as numerous as the homozygous class has a greater chance to be obtained from the goldfish breeders. Another explanation is that the goldfish breeders often put five or six fishes into a breeding basin and frequently a normal scaled fish is mated with the transparent scaled fish with the result that most of the transparent scaled offspring are heterozygous. A third explanation is that during the classification and counting of the fishes of the F_4 generation containing the three different kinds of fishes I noticed that when the number of fishes in a basin amounted to several hundred with a keen competition of the fishes in the struggle for food, the transparent homozygous fish were always smaller than the other two classes of fish. The common practice of the goldfish breeder is to make a selection of the desirable fishes to rear when the young fishes reach the size of about 20 to 30 mm. At this time the larger fishes are selected to be grown for sale or for breeding and the smaller fishes are discarded or disposed of in other ways. If the transparent fishes are allowed to grow along with other fishes, it is probable that the other classes of fish will be selected in greater number and the transparent homozygous fish will be mostly discarded and only a small portion selected and grown for sale or for breeding. Probably, it is due to all the above mentioned reasons that the homozygous transparent fishes are rare and difficult to obtain from the common goldfish dealer and the existence of this class of fish has not yet been recorded in the goldfish books of any language.

SOMATIC MUTATIONS

In the summer of 1926 I found that among the offspring of the matings

between the normal scaled females and males there were six fishes which had a portion of their scales becoming transparent. But these fishes were different from the transparent scaled fishes which I studied. In the ordinary transparent scaled fishes the homozygous forms are nearly completely transparent and without chromatophores and the heterozygous fishes have the characteristic mottled pattern while in the case of the exceptional transparent scaled fishes produced from the mating of two normal scaled fishes the reflecting tissue is only partially reduced and yet the chromatophores do not form the characteristic mottled pattern. These exceptional transparent scaled fishes were of the ordinary red or white color like ordinary normal scaled fishes.

In the spring of 1927 I made two matings of the exceptional transparent scaled fishes with normal scaled fishes. These two matings (E3, E5) produced only normal scaled offspring. In the same breeding season I made one mating (1-9) of the exceptional transparent females and the exceptional males. The offspring obtained from this mating were also all normal scaled as the other normal scaled breeds of goldfish. This result proved that these exceptional transparent scaled fishes were genotypically with the TT genes but phenotypically with some transparent scales. Probably the formation of the transparent scales on the body of such fishes might be due to a somatic mutation of one of the gene T into a gene T' but the germplasm was not affected.

Among the forty fishes which I used to distinguish the homozygous from the heterozygous fish by means of the progeny tests I found one exceptional female fish ($B11$) which had one side of its body like an ordinary transparent homozygous fish but another side of its body like an ordinary heterozygous mottled fish with a non-transparent operculum and three normal scales, a thing which has never been found in the ordinary homozygous transparent fish. The mating of this fish with a normal scaled male fish produced offspring all belonging to the heterozygous mottled class thus proving that the fish was homozygous for the two $T'T'$ genes. The fact that one side of the body showed the heterozygous condition might be explained by a somatic mutation of one of the T' genes into a T gene during the early stage of development of the fish so that one side of the body was affected.

If the explanations of the above exceptional cases be true we might say that the gene T may mutate to gene T' in the somatic cells and the reverse mutation is also possible.

REVIEW OF LITERATURE AND COMPARISON WITH OTHER CASES

So far as I know there is only one paper on the inheritance of the characters of goldfish. This is the paper of HANCE (1922). In this paper HANCE touched upon the inheritance of colors, telescopic eyes, and the paired caudal fins and concluded, without reporting any data, that these characters were Mendelian. Although there was a picture of the mottled fish in his paper he seemed not to have worked or noticed the simple inheritance of this character. In my breeding work I found the inheritance of the color, telescopic eyes, and paired caudal fin, although with segregations in the F_2 generation, was very complicated whereas the inheritance of the mottled pattern was very simple and followed Mendel's law with the observed ratio very close to expectation. Probably this is the first case of Mendelian inheritance observed in the ordinary goldfish.

The inheritance of the transparency and mottling is a peculiar case of Mendelian inheritance in several respects. The heterozygous condition is a new type distinct from the two homozygous types. The new heterozygous character is a mosaic of the two homozygous types in regard to the presence and absence of reflecting tissue and chromatophores. The genes produce manifold effects on the reflecting tissue, melanophores, and xanthophores. A variegated condition is produced by the contrasting allelomorphic genes. Expression of the genes is somewhat variable. The combination of the above cited peculiarities in a single case is very rare. I found no parallel case in the genetics of animals or plants.

The case of the transparency and mottling is similar to that of the blue Andalusian fowl as reported by BATESON and PUNNET (BATESON 1911). In each case there is a distinct heterozygous type different from the two homozygous types the ordinary distinction of dominance and recessiveness being lacking. The heterozygous type in both cases is a sort of mosaic of the two homozygous types. In the goldfish the heterozygous type is mosaic in regard to the presence or absence of reflecting tissue and chromatophores. In the fowl, the heterozygous type is mosaic in regard to presence and absence of black pigment. However these two cases are not entirely similar since the genes of the goldfish have manifold effects while those of the fowl have only a single visible effect.

The case of transparency and mottling is more similar to that of the roan in the shorthorn cow (BATESON 1911). Here the red color in crossing with the white cow produces offspring with both red and white spots or roan just as the crossing of fishes with reflecting tissue distributed uniformly on the body with fishes lacking the reflecting tissue produces off-

spring with variegation in the presence or absence of the reflecting tissue. These two cases are also similar in the mosaic expression of the two homozygous types. However they are dissimilar in the presence of the manifold effects in the case of goldfish and the absence of it in the case of cattle.

SUMMARY

1. A pair of Mendelian factors has been definitely found in the goldfish. I propose to call the new character "transparent." This pair of factors is not sex-linked.

2. The homozygous condition of the transparent factor has the following manifold effects: the reflecting tissue is nearly lacking so that the red gills are visible through the operculum, the black retinal pigment is visible through the iris, and the white testis or yellow eggs are visible through the body walls. In such a fish the melanophores are restricted to a few black dots aggregated near the junction between the caudal peduncle and the caudal fin. The xanthophores are generally completely absent. The normal allelomorph of transparent when homozygous causes the production of the reflecting tissue and the two kinds of chromatophores to cover the whole body.

3. The heterozygous condition causes the production of the type of goldfish called "calico" in the United States and "shubunkin" in Japan. This heterozygous form is a mosaic of the two forms of homozygous fish in regard to the reflecting tissue, but the combined action of the two kinds of genes affects the chromatophores in a peculiar way so that a characteristic mottled pattern is produced.

4. In a strict sense there is no dominance and recessiveness between the members of this allelomorphic pair. But, according to the idea of MORGAN, the transparent condition might be said to be dominant and the normal condition to be recessive because the heterozygous mottled condition is sharply distinct from the normal form but is somewhat overlapping with the homozygous transparent condition especially when the fish are smaller than 20 mm in size.

5. Some cases of somatic mutation of the normal gene into the transparent gene have been observed and a case of somatic mutation of the transparent gene into the normal gene has also been observed.

6. Wild goldfishes have been successfully crossed with different kinds of fancy goldfish with the production of fertile offspring.

The above cited investigation was undertaken in the Biological Laboratory of the SCIENCE SOCIETY OF CHINA while I was occupying a position in the Department of Zoology in the College of Agriculture of the NATIONAL

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